

School of Biomedical Sciences

**Determination of the Mutagenic Potential of Commonly Marketed Ghanaian
Medicinal Plant Formulations Using the Bacterial Reverse Mutation Assay**

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**This thesis is presented for the Degree of
Master of Philosophy (Biomedical Sciences)
of
Curtin University**

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This thesis is dedicated to my parents, Emmanuel and Victoria Senayah and to my lovely siblings: Florence, Raymond, Saviour, Juliet, Matilda and Angela. I got this far because you believed in me.

DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Fidelia Senayah

January 2015

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ABSTRACT

Herbal medicine is widely used, especially in developing countries because it is readily available and affordable. In Ghana, most people depend on herbal medicine to meet their basic healthcare needs. Though herbal medicine has been in use over a long period of time, sufficient scientific data supporting the claimed therapeutic benefits of medicinal plant formulations for human ailments is limited. Whilst much of the global research efforts on the therapeutic potential of medicinal plants focus mainly on the screening for phytochemical constituents, antimicrobial efficacy, and toxicity, there is the need to investigate not only potential benefits but also deleterious effect of these herbal medicinal products on human health.

The major aim of this investigation was to screen commonly marketed herbal medicinal products on the Ghanaian market for their mutagenic potential using the bacterial reverse mutation assay (Ames test). The *Salmonella typhimurium* mutant strains TA98, TA100 and TA1537 were employed using 2 different concentrations (1mg/mL and 8mg/mL) of the herbal formulations based on their protein and carbohydrate content. The test was carried out using induced (S9-activated and Rifampin-activated S9 microsomal fractions prepared from adult pig liver). The antimicrobial properties of commonly marketed Ghanaian medicinal plant product formulations.

Results of the mutagenicity study showed that none of the products tested indicate any positive mutagenic effect on the mutant strains tested. On the other hand, one of the extracts, E8 containing a combination of aqueous extracts from *Clausena anisata*, *Pilostigma thonningii*, *Trichilia monadelpha*, *Khaya senegalensis* and *Nauclea latifolia* showed a 2-fold decrease in revertant colonies compared to that of the negative controls for all strains in the metabolic activation system of the assay, indicating the potential antimutagenic activity of the combined extract. Extract E8 also showed bactericidal activity against *Moraxella catarrhalis*, a causative agent of otitis media in children, particularly affecting the Australian Indigenous community. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration of E8 against *M. catarrhalis* was 125µg/mL and 500µg/mL respectively. None of the other extracts showed antimicrobial activity against the pathogens tested in this investigation.

It thus appear, that one or more of the plant constituents in E8 formulation may contain bioactive compounds responsible for the observed antimicrobial activity. Further investigations to determine the plant constituents of E8 contributing to the antimicrobial and antimutagenic activities are clearly warranted.

STATEMENT OF CONTRIBUTION

I hereby declare that work submitted for publication and presented in this thesis was designed, written, experimentally conducted and interpreted by myself, Fidelia Senayah.

Chapters 1 to 7 – These chapters were reviewed by Associate Professor TK Mukkur, providing critical input.

Chapter 3 and 4 – Heather Fairhurst and Alain Delhaize provided technical assistance with the Ames test laboratory procedures and Associate Professor Robert Tuckey of the University of Western Australia provided the facility, equipment and assistance with the quantification of the Cytochrome P450 content of the microsomal fractions prepared from adult pig liver.

Chapter 5 – Alain Delhaize provided the microbial isolates for the testing of antimicrobial activity of the medicinal plant formulations and some of the antibiotics for developing the antibiograms. Abdulamer Al-Waely provided technical assistance in the determination of antimicrobial activity of the medicinal plant formulations.

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The aims/objectives of the study are as follows:

- ❖ Screen commonly marketed herbal medicinal products on the Ghanaian market for their mutagenic potential using the bacterial reverse mutation assay (Ames test)
- ❖ Determine the antimicrobial properties of commonly marketed Ghanaian medicinal plant products using a multiplicity of pathogens.

Outline of thesis

This thesis has been prepared using a hybridization format of submitted and/or prepared manuscripts, with individual chapters on introduction and background review, materials and methods, supplementary results for Ames test, general discussion, conclusion/future directions/study limitations and references.

CHAPTER 1 – (1.1 and 1.2) Introduction and background review of Ames test

CHAPTER 1 - 1.3 : In this section of the chapter, a comprehensive review of literature of the individual plant constituents of the nine Ghanaian medicinal plant formulation was undertaken. This consisted of their general description, traditional uses, phytochemical constituents, antimicrobial activities, anti-parasitic activities, pharmacological studies, cytotoxicity and genotoxicity studies.

CHAPTER 2 – General Materials and Methods that could not be accommodated in the manuscripts are described in this chapter.

CHAPTER 3 – The mutagenic potential of nine (9) commonly marketed Ghanaian aqueous medicinal plant formulations were tested against *Salmonella typhimurium* mutant strains TA98, TA100 and TA1537 at a concentration of 1mg and 8mg/mL based on their protein and carbohydrate concentrations using Ames test. This test was performed for both induced (S9 activated and S9+Rifampin activated) and un-induced (without metabolic activation) pre-incubation method. None of the extracts tested positive for mutagenicity. However, one extract, E8 was found to produce a 2-fold decrease in revertant colonies as compared to the negative control, indicating antimutagenic activity of product E8.

CHAPTER 4 - Supplementary information for herbal extracts demonstrating negative outcomes in the Ames test for all *Salmonella typhimurium* strains which were not submitted for publication is presented in this chapter.

CHAPTER 5 - Antimicrobial activity of the Ghanaian medicinal plant products, performed using agar well diffusion and microdilution method for minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) as described. The extracts were tested against *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Moraxella catarrhalis*, *Bordetella parapertussis*, *Acinetobacter baumannii*, *Salmonella typhimurium* and *Candida albicans*, at 20mg/mL. Of the nine extracts tested, Extract E8 showed bactericidal activity against *Moraxella cataarhalis* with an MIC of 125µg/mL and MBC of 500µg/mL.

CHAPTER 6 – General Discussion

CHAPTER 7 – Conclusion and future directions

REFERENCES – This consist of all references cited in this thesis

LIST OF ABBREVIATIONS USED IN THIS THESIS

CDS	Calibrated dichotomous test
DMSO	Dimethyl sulfoxide
INT	p-iodonitrotetrazolium violet
DPPH	2,2, diphenyl 1-1 picrylhydrazyl
XTT	2,3-Bis(-2-methoxy-4-nitro-5-sulfophenyl) -2H-tetrazolium-5-carbozanilide
MTT	3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide
TAC	Total antioxidant capacity
ABTS	Thiobarbituric acid reactive substances
LD₅₀	Lethal dose, 50%
IC₅₀	Half maximum inhibitory concentration
LC₅₀	Lethal concentration, 50%

Introduction and Literature Review

1.1 Introduction

Herbal medicine is “a plant- derived material or preparation with therapeutic or other human health benefits which contains either raw or processed ingredients from one or more plants” (World Health Organization (WHO, 1993). Many plant species used in herbal medicine have been found to contain active compounds, which can be extracted and used as traditional drug alternatives. The plant can also be used either directly or as an extract (crude extract) for medication, a practice which is common in developing countries (Ishii et al., 1984; Hoyos et al., 1992). Herbal medicine plays a significant role in Africa, and has also maintained its popularity worldwide. About 80% of African and Asian populations, including Ghana, depend on herbal remedies as alternative to orthodox medicine for their primary health care. Even in many developed countries, 70% to 80% have used some form of alternative or complementary medicine at least once (WHO, 2008). Herbal medicine is also the first line of treatment for about 60% of children with malaria in Countries like Ghana, Nigeria, Zambia and Mali (WHO, 2003), because of the easy and affordable access to herbal medicine.). In Ghana, it is estimated that 951 tons of crude plant medicine (excluding pre-packed herbal medicine) are sold annually on the Ghanaian domestic market, amounting to a total of US\$7.8 million market value and an export value of US\$15 million (Van Andel, Myren and Van Onselen, 2012). Medicinal plants have been used as antimicrobial, antidiabetic, anti-atherogenic, antioxidants, anti-cancer and anti-malarials (Afolayan and Sunmonu, 2010; Afriyie et al, 2013; Aquil, Ahmed and Mehmood, 2006; Azas et al, 2002; Boakye-Yiadom, 1979 Khan et al, 2012; Sawadogo et al, 2012; Tempesta, 2010).

Based on their long-term use without adequate scientific research, many people believe that herbal medicines are safe and have either low or no toxicity. However, some herbal medicines can have harmful adverse reactions due to the presence of toxic constituents (Zink and Chaffin, 1998). In some African countries, herbal medicines have been reported to be responsible for 0-35% of cases of acute renal failure (ARF), (Foyaca-Sibat, Ibañez-Valdès and Awodetu, 2001). For instance in

Kenya, about 10.9% of all cases of ARF over a period of two years was attributed to the use of herbal remedies (Otieno, McLiqeyo & Luta, 1991). Chinese herbs containing the plant ingredient aristolochic acid has also been suggested to cause urinary bladder cancer and end-stage kidney failure (Cosyns, 2003; Laing et al, 2006). Other research findings have also indicated some plant extracts to be mutagenic (Wright et al 2001; Ansah, Khan & Gooderham, 2005; Rojas, Wright, Pina & Portugal, 2008; Gopalan et al, 2011) and cytotoxic (Higashimoto et al., 1993; Haq, 2004; Plewa and Wagner, 1993), raising some concern about the long-term use of such medicinal plants.

The aim of this project was to screen commonly marketed herbal medicinal products on the Ghanaian market for their mutagenic potential using the bacterial reverse mutation assay (Ames test). The assay is specifically designed to detect the mutagenicity of a wide range of compounds and biological molecules capable of producing genetic damage leading to gene mutations. Validation tests have also shown a high predictive value for rodent carcinogenicity with a positive Ames test (MaCann and Ames, 1976; Zeiger et al., 1990). The test was be carried out with and without metabolic activation, since some potential mutagens need to be metabolized in the liver to produce biologically active potential carcinogens.

1.2 Background Literature Review of Ames test

1.2.1 Ames test

The Salmonella/microsome reverse mutation assay, referred to as Ames test, was previously developed by Bruce Ames (1971) to detect the mutagenic potential of chemical substances, as well as a wide range of environmental and biological compounds in mixtures (Maron and Ames, 1983). Chemical mutagens detected by the Ames test have subsequently been shown to be carcinogenic in animals, and carcinogens also found to be mutagens. These include aflatoxin B₁, mitomycin C, daunomycin, fungal toxins, and cigarette smoke condensate (Ames, McCann and Yamasaki, 1975; Ames, 1972; Ames, Lee and Durston, 1973); Maron and Ames, 1983; Mortelmans and Zeiger, 2000). The Ames test is also used as an initial screening test worldwide for the registration of new drugs and chemicals by regulatory agencies (Maron and Ames, 1983; Mortelmans and Zeiger, 2000). The test employs *Salmonella typhimurium* auxotrophs, which are histidine deficient mutant strains of *Salmonella typhimurium*. Each strain has different type of mutation in the histidine operon. In addition to this, the strains have DNA repair mutation in the *rfa* genes determining biosynthesis of somatic lipopolysaccharide core of the *S. typhimurium* strain, with or without the presence of plasmid pKM101. This plasmid imparts ampicillin resistance and also the ability of the test to detect mutagens as a result of reverse mutation of *rfa* genes (Maron and Ames, 1983).

Table 1

Characteristics of *Salmonella typhimurium* strains

<i>Salmonella</i> Strain	Gene Affected	DNA Repair	LPS	Biotin Requirement	R Factor Plasmid	Mutational Event
TA98	hisD3052	uvrB	rfa	bio-	pKM101	Frameshift
TA100	hisG46	uvrB	rfa	bio-	pKM101	Base-pair substitution
TA1537	hisC3076	uvrB	rfa	bio-	-	Frameshift

1.2.2 Properties of *Salmonella typhimurium* auxotrophs

1.2.2.1 DNA repair mutation

The *uvrB* gene, which encodes *uvrB* protein, that is induced by UV light and some chemical mutagens, which in coordination with *uvrA* and *uvrC*, causes damage to DNA repair mechanisms. The presence of this mutation makes the auxotrophs more sensitive to chemicals capable of inducing damage. The mutation also extends to the gene required for biotin synthesis, hence the need to add biotin to the test system (Hsu et al, 1995). The *uvrB* mutation is indicated by sensitivity to UV light.

1.2.2.2 *rfa* mutation

Salmonella typhimurium strains defective in the *rfa* gene result in defective lipopolysaccharide layer of the bacterial cell, making the outer membrane of the cell more permeable to large molecules (Parry and Parry, 2012). The presence of *rfa* mutation is indicated by sensitivity to crystal violet.

1.2.2.3 R factor plasmid (pKM101)

Plasmid pKM101 is a conjugate plasmid that codes for transmissible drug resistance. pKM101 is derived from R46 by deletion of a single region of DNA. The plasmid codes for β -lactamase that makes the strains resistant to ampicillin. It increases cellular survival after UV radiation, chemical and spontaneous mutagenesis (Walker et al, 1975).

1.2.2.4 Histidine Biosynthesis Pathway

Salmonella typhimurium wild-type strains are able to grow on culture media without the addition of amino acids. This is due to the availability of enzymes required for histidine synthesis in the pathway. *Salmonella typhimurium* auxotrophs on the other hand are mutants, *his*⁻ (histidine-dependent) due to mutations in a gene which is required in the production of histidine. The strains have G-C base pairs at their site of reversion, thus, -C-C-C in base pair substitution and -C-C-C-C-C-C- or -C-G-C-G-C-G-C-G in frameshift strains (Levin et al, 1982). The *his*⁻ auxotrophs require an external source of histidine to grow unless there is a reversion of the mutation which will allow histidine production. The histidine biosynthesis pathway is composed of

ten enzymatic steps and eight genes. Three out of the eight genes, *hisD*, *hisB* and *hisI*, encode bifunctional enzymes and two other genes, *hisH* and *hisF* encode polypeptide chains. Figure 1 shows the histidine biosynthesis pathway (Alifano et al, 1996).

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The manuscript is attached on the next page.

A Review of the Biological Activities and Traditional uses of Plant Constituents in some Ghanaian Medicinal Products

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Abstract

Medicinal plants are widely used, especially in developing Countries. These medicines have become an important part of the primary health care delivery system, hence their use in the treatment of many ailments. In Ghana, as is the case with most other developing countries, many people depend on herbal medicine to meet their basic health care needs because of ease of accessibility and affordability. This review aims at providing update on the traditional uses and biological activities of medicinal plants found in commonly marketed herbal medicines in Ghana.

Keywords: Medicinal plants, biological activities

Introduction

Herbal medicines play significant role all over the world. Most African nations are endowed with rich natural plant vegetation and these plants have been in use since ancient days. Many plant species used in herbal medicine have been found to contain active compounds, which can be extracted and used as traditional drug alternative. The plant can also be used either directly or as an extract (crude extract) for medication, a practice which is common in developing countries (Ishii et al., 1984; Hoyos et al., 1992). The World Health Organization (WHO) estimates that about 80% of African and Asian population relies on herbal remedies as alternative to orthodox medicine. It is also estimated that in Ghana, herbal medicine is the first line of treatment for children with malaria (WHO, 2003). Medicinal products sold on the

Ghanaian markets are usually in the form of finished products which are indicated for the treatment of common ailments such as malaria, typhoid fever, haemorrhoids, management of diabetes and hypertension among others. These products may be prepared from one plant or combination of two or more plants. However, raw plant parts are also readily available and sold on the market as well. The main objective of this review is to highlight the biological activities of individual plants commonly found in medicinal products sold on the Ghanaian Market.

Medicinal Plants Commonly used in Ghana and Their Biological Activities

***Nauclea latifolia* (Sm)**

Description: *Nauclea latifolia* belongs to the family Rubiaceae and is mostly found in tropical Africa and Asia but also cultivated worldwide. It is commonly called African peach or pin cushion tree. Its local Ghanaian names are: Paye biasa, kisia, oyefa, Kankanu (Twi), ekusiawa (Fante), Nyimo (Ewe), Oyeta-owefa (Ga), Gulungun (Dagbani). It is a straggling multi-stemmed shrub or small tree that grows up to about 4m high. The leaves are broadly elliptic to rounded-ovate, bark rough, flowers white or yellowish white and fragrant, fruit reddish [4].

Traditional Uses: Almost all parts of *N. latifolia* are used in traditional medicine for therapeutic benefits. In West and South Africa, the leave and bark are used in the treatment of diarrhea, malaria and stomach pains ([5], [6]). In Nigeria, it is used as a remedy for stomach ache and tuberculosis [7]. In Ghana, the roots, bark and leaves are also used for the treatment of arthritis, malaria, oliguria, halitosis, infective hepatitis and jaundice [4].

Phytochemical Constituents: Phytochemical analysis of aqueous and methanol extracts of the leaves, stem bark and roots has shown the presence of saponins, alkaloids, glycosides tanins and flavonoids [8].

Antimicrobial Activity: The methanol, hexane and aqueous extracts of leaves and stem bark of *N. latifolia* is reported to have inhibitory effects on wild strains of *Escherichia coli*, *Streptococcus pneumoniae*, *Shigella dysenteriae* and *Staphylococcus aureus* [8]. Other broad spectrum antimicrobial activities have also been observed ([9], [10], [11]). Wound healing and antidiarrheal activity of the stem and root backs was also observed in rabbits and diarrhea induced rats [12].

Pharmacological Studies: Extract of the plant is reported to significantly reduce, the level of plasma cholesterol in albino rats, an indication of its hypocholesterolemic effect [13]. The root and stem have also been shown to antihypertensive properties, reducing serum sodium and chloride levels in male and female albino Wister rats; the root extract reduced systolic, diastolic and mean arterial pressure in normotensive and hypertensive rats ([14], [15]). Ngo et al., 2009 [16] reported the sedative, anticonvulsant and anxiolytic-like effects of the root bark in animal models. The root bark also showed antipyretic, antinociceptive and anti-inflammatory effects in mice and rats ([17], [18]). It reduced uterine contraction induced by oxytocin, acetylcholine and ergometrine in non-pregnant female albino rats [19], thus supporting its use as anti-abortion. The root also exhibited antidiabetic activity in STZ-induced diabetic rats [20]. However, the aqueous extract of the stem has hepatotoxic and nephrotoxic potential [21].

Anti-parasitic Activity: *N. latifolia* has been reported to possess shown antiplasmodial activity against both chloroquine-resistant and chloroquine-sensitive strains of *Plasmodium falciparum* ([22], [23]). The ethanol root extract is also reported to have decreased the level of parasitaemia in mice experimentally infected with *Trypanosoma brucei brucei* [24]. Makai and Kobo, 2008 [25] reported a similar anti-trypanosomal activity of the stem bark on *Trypanosoma congolense*. The leaf extract killed the infective larvae stage of gastrointestinal nematodes in sheep [26].

Genotoxic and Cytotoxicity Studies: Saponins from the hydromethanolic extracts of *N. latifolia* was observed to exhibit genotoxic activity in the micronucleus and comet assays by inducing synergistic DNA-damage *in vitro* and chromosome mutations in mammalian cells [27]. Alkaloid rich chloroform extract of the plant is also reported to be mutagenic in the presence of metabolic activation with *Salmonella typhimurium* mutants TA97 and TA100. It induced single-strand breaks in human lymphocytes [6]. Relatively low cytotoxicity in different cell lines has also been reported Zirihi et al., 2005 and Ajaiyeoba, 2006 ([23], [28]).

***Phyllanthus fraternus* (Webster)**

Description: *Phyllanthus fraternus* belongs to the family Euphorbiaceae. It is native to India, Amazon rainforest and also available in other tropical Countries. In Ghana it is called Nambu (Hausa), Ofobiokpai (Dangme), Adfiehotsui (Ewe), Nkatsena

(Fante) Awomma agu wakyi, Awobe (Twi). It is a glabrous annual herb which grows up to about 30-40cm high. The stem is smooth with slightly winged branchlet. Leaves elliptic, round at both ends ([4], [29]).

Traditional Uses: *P. fraternus* is used traditionally for the treatment of fever, dysentery, diabetes, jaundice, as a diuretic, stomach ache ([30], [31]), gynaecological disorder [32], viral infections, wound, fracture [4], liver, intestinal and bladder disorders [33].

Phytochemical Constituents: The plant contains reducing sugars, glycosides, steroids, alkaloids, tanins, saponins and flavonoids [33].

Antimicrobial Activity: Extracts of *P. fraternus* has been reported to be effective against clinical (wild) isolates and World Health Organization (WHO) strains of *Neisseria gonorrhoeae* [34].

Pharmacological Studies: Aqueous extracts of *P. fraternus* has been shown to have significant protective effect on the mitochondrial dysfunction of rats induced with carbon tetrachloride [35]. Similar hepatoprotective effect against carbon tetrachloride and galactosamine induced toxicity has been demonstrated by Khatoon et al., 2006 [33]. *P. fraternus* has been reported to have protective effect against cytotoxicity induced by lead and aluminum salts [36]. The plant extract produced significant inhibition of capsaicin-induced neurogenic pain, formalin-induced pain and acetic acid induced abdominal constriction in Swiss mice [37]. It also showed protective effect against superoxide radicals and alcohol-induced oxidative stress in liver mitochondria of rats. The ability of the plant to prevent or reduce deterioration caused by free radicals was also demonstrated by Naga et al., 2008 [38] and similar antioxidant activity has also been reported by Koffuor and Amoateng, 2011[39].

Cryptolepis sanguinolenta

Description: *Cryptolepis sanguinolenta* belongs to the family Periplocaceae. It is mostly found in tropical West Africa. It is also known by local Ghanaian names such as Kadze (Ewe), Nibima (Twi) and Gunngamau (Hausa). It is a tropical shrub with thin branches measuring up to 8m high. Leaves are oblong-elliptic, ovate, acutely and shortly acuminate. The leaves and roots are yellow in appearance ([4], [40]).

Traditional Uses: Aqueous extracts of *C. sanguinolenta* are traditionally used to treat acute malaria, hypertension and inflammatory conditions [41]. In Ghana, it is

commonly used in the treatment of fever, malaria, insomnia, urinary and upper respiratory tract infections [42].

Phytochemical Constituents: Phytochemical screening of aqueous and ethanol extracts has showed the presence of alkaloids, reducing sugars, polyuronides, and anthocyanosides [43].

Antimicrobial Activity: Aqueous and organic of *C. sanguinolenta* root have been reported to have exhibited antibacterial activity against both methicillin-resistant and methicillin-sensitive [44]. Cryptolepine, an indoloquinoline alkaloid of *C. sanguinolenta* has been shown to have activity against *S. aureus*, *E. coli*, *Candida albicans*, *Saccharomyces cerevisiae* and *Campylobacter species* and *Vibrio cholera* ([45], [46], [47]). Cryptolepine hydrochloride, a salt of the alkaloid also inhibited the growth of *Mycobacterium* species suggesting its anti-mycobacterial properties [48]. Other fractions of *C. sanguinolenta* extract have also shown antimicrobial properties ([49], [50], [43]).

Antiplasmodial Activity: Both *in-vitro* and *in-vivo* studies on *C. sanguinolenta* have shown its effectiveness in the treatment of malaria. Clinical study of the efficacy of a tea-bag formulation in patients with uncomplicated malaria showed the ability of the extract to clear parasitaemia in over 50% of patients within 72 h ([51], [52]). Cryptolepine was highly active against multidrug resistant strains (KI) of *P. falciparum* [53]. 2-Bromoneocryptolepine and other derivatives of neocryptolepine, an alkaloid of *C. sanguinolenta* has been shown by Jonckers et al., 2002 [54] to have antiplasmodial action against chloroquine-resistant *P. falciparum* (Half maximal inhibitory concentration, IC₅₀ 4.0µM). Cryptolepine derivatives administered orally to mice infected with *P. berghei* showed reduction of parasitaemia by 89% with the most potent compound being 2, 7-dibromocryptolepine [55]. Other antiplasmodial activities have been reported by Addae-Kyereme 2004 and Rocha et al., 2012([56], [57]).

Pharmacological Studies: Cryptolepine and its salts; hydroiodide, hydrochloride and hydrotrifluoro-methanesulfonate have been reported to have lowered blood glucose in rodent models with type II diabetes [58]. The effect of aqueous root extract on reproductive and foetal development in mice has also been studied by Ansah et al., 2002 [59]. The extract was reported to have reduced female fertility

(100% to 0%) and terminated pregnancy in 60% of mice treated during gestation, with 37.0% intrauterine growth inhibition and 12.0% foetal mortality. The alkaloids, cryptolepine and 11-hydroxycryptolepine, have been reported to possess antimuscarinic and antioxidant properties ([60], [61]).

Genotoxicity and Cytotoxicity Studies: Cryptolepine is reported to exhibit cytotoxic effects on P388 human leukemia cell lines [62]. It also induced mutation at the hprt locus and micronuclei formation in V79, a Chinese hamster lung fibroblast cell line [63]. DNA damage in human lymphocytes caused by cryptolepine and its analogues has been reported by Gopalan et al., 2011 [64]. The potential of the alkaloid to intercalate into DNA promoting DNA lesions was also observed in *S. cerevisiae* [65]. There are also similar findings on cytotoxicity/DNA intercalating activity of cryptolepine and its derivatives ([66], [54], [57]).

Ocimum gratissimum

Description: *Ocimum gratissimum* is a member of the family Lamiaceae (Labiatae). It is native to tropical Africa, Southern Africa, Western Indian Ocean and Asia. It is commonly called basil or tea bush. Other local names are Nunum, onunum (Twi), Bebusui, Dzeveti (Ewe), Sulu (Ga), Dardoyatagida (Hausa). It is a perennial aromatic herb, with stem measuring about 1-3m long. It is round-quadrangular, branched and globorous or pubescent. The leaves are broad, 5-13 cm long and 3-9 cm wide [67].

Traditional Uses: *O. gratissimum* is used traditionally to treat upper respiratory tract infections, skin diseases, cough, diarrhea, conjunctivitis and headache [68]. It is also used for the treatment of malaria, dyspepsia, rheumatism, dysentery, sinusitis, fever and snake bites [4].

Phytochemical Constituents: Phytochemical analysis has shown the presence of saponins, steroidal terpenes, glycosides, flavonoids, alkaloids, resins, and tannins [69].

Antimicrobial Activity: The essential oil of *O. gratissimum* is reported to have antibacterial activity against *Shigella flexneri*, *S. enteritidis*, *E. coli* and *Klebsiella* species. Leaf extracts also showed activity against *Proteus mirabilis*, *S. aureus* and *E. coli* ([70], [71]) and other silimar clinical isolates ([72], [69], [73], [74]). The extract is reported to have anti-diarrhoeal effect on castor oil induced diarrhea in rats [75] and clinical isolate [68]. Its ability to increase vascular permeability, hence

wound healing was observed in rabbits [76]. Kishore et al., (200), [77] demonstrated the activity of *O. gratissimum* against *Scopulariopsis brevicaulis* which causes skin mycosis and *Cryptococcus neoformans* which is common in HIV infected patients. Other antifungal activity has been shown in *Candida* species [78]. The Chloroform fraction (92%) and eugonol (16%) inhibited the activity of clinical fungal isolates [79]. The activity of leaf extract on *in vitro* replication of HIV-1 and HIV2 is reported by Ayisi and Nyadedzor 2003, [80].

Anti-parasitic Activity: The essential oil and eugonol had inhibitory effect on the growth of the *Herpetomonas samuelpeessoai*, a trypanosomatid at a concentration of 20 to 250µg/ml [81]. It also inhibited the growth of *Leishmania amazonensis* ([82] and *Haemonchus contortus*, a causative agent of gastrointestinal helminthiasis of goats, small ruminant animals [83]

Pharmacological Studies: The leave extract of *O. gratissimum* is reported to have significantly lowered, plasma glucose level in streptozotocin induced diabetic rat [84]. This results correlates with earlier work by [85], where *O.gratissimum* extract reduced plasma glucose levels in both normal and alloxan induced diabetic rats by 56 and 68% respectively. The essential oil and eugonol induced hypotensive effect in hypertensive rats. It also relaxed phenylephrine-induced contraction in isolated aorta preparations with intact endothelium from rats [86]. A relaxant effect on intestinal smooth muscle of guinea pig was also reported by Madeira et al., 2002, [87]. Reference [88] observed the protective effect of the methanol extract of *O. gratissimum* against ethanol induced hepatotoxicity in male Wistar rats. Other studies on acute, sub-acute and gastric tolerance of the oil extract in Wistar rats given at a dose higher than 1500mg/kg body weight did not show any adverse effects in the rats [89]. Previous toxicity study of the essential oil in rats and mice showed a dose-dependent sedative effect [90]. The study also demonstrated an inflammatory response upon persistent administration of the oil in the rats and mice. An anti-nociceptive effect of the essential oil of the plant has been studied in two models of pain in Swiss male mice. The oil showed a dose dependent inhibition of acetic acid induced writhing, causing up to a 60% inhibition at the highest dose of 300mg/kg (po). At the same dose range (30, 100 and 300mg/kg), it predominantly inhibited the late (inflammatory) phase of formalin induced pain response ([91]. Akinmoladun et al., 2007, [92] demonstrated 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical

scavenging activity of 84.6% at 250µg/ml and a reduction potential of 0.77 at 100µg/ml using methanol extract. DPPH scavenging by both polar and non-polar extracts was also reported by Oboh et al., 2008, [93].

Genotoxicity and Cytotoxicity Studies: *Ocimum. gratissimum* has been associated with anti-carcinogenic and chemo-preventive activities [94]. Organic solvent extract of the leaves was shown to be antimutagenic. It inhibited the activity of His⁻ to His⁺ reverse-mutation induced by ethyl methanesulfonate acting on *Salmonella typhimurium* TA100 [95]. The plant has also been reported by Nangia-Makker et al., 2007, [96] to have inhibited tumour growth and angiogenesis.

Cnestis ferruginea

Description: *Cnestis ferruginea* (Vadhl) D.C belongs to the family Connaraceae. It is vastly abundant in Western part of Africa and other tropical regions [97]. Its local Ghanaian names are: Apose, Apowse (Twi), Akitase (Fante), Pudaegye (Nzema). It is a shrub of 3–4m in height. Its branches rusty brown. Leaves are indeciduous, 4–16-jugate with petiole 2–10 cm, rachis 12–27 cm long and densely rusty brown pubescent. Lateral leaflets are more or less alternate or sometimes opposite, ovate to narrowly oblong, slightly or not unequal at base, and terminal leaflet is elliptic or narrowly ovate ([98], [99]).

Traditional Uses: Aqueous decoction of the roots is used to treat headache, urethral discharge, migraine, toothache, skin infections, sinusitis and gynaecological conditions [97]. The leaves are used as laxative, for cough, anaemia and for wound infections ([99], [4]). Preparations from the bark are also used to treat infected gum and infantile diseases, while the fruit and roots are also used as remedy against snake bites [100].

Phytochemical Constituents: Phytochemical analysis has shown the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones, phenols, glycosides and steroids [101].

Antimicrobial Activity: Extracts of the root, stem bark and leaves tested against clinical isolates showed susceptibility of most gram positive bacteria including β-haemolytic group A *Streptococcus* and *Bacillus cereus* as well as antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and other clinical isolates ([102], [103]).

Pharmacological Studies: Alkaloid crude fraction of the root has been reported to have adverse effect on hepatic function of male Wistar rats. Administration of the fraction reduced alkaline phosphatase (ALP) and aspartate transaminase (AST) levels; there were increases in serum albumin, conjugated and total bilirubin. Histological examination also showed severe disruption of the liver structure and presence of red blood cells in hepatocytes [104]. Methanol root extract of the plant has been reported by Ishola et al., 2012 [105] to have insignificant effect on the weight and vital organs of albino rats. Hepatoprotective effect of the leaf extract was also observed in paracetamol induced Swiss albino mice [101]. Toxicity due to the presence of compounds dicoumarol and 4-hydroxycoumarin in rabbits has also been reported by Vickery and Vickery, 1980 [106]. Purified methanol fractions caused leucopenia and in some cases anaemia [107]. Oral administration of ethyl acetate extracts (250mg/kg body weight) to streptozotocin (STZ)-induced diabetic rats and mice showed a significant reduction in fasting blood glucose (FBG) levels and also lowered serum creatinine, total cholesterol, triglycerides and thiobarbituric acid reactive species levels. Serum ALT and AST levels were also significantly reduced [108]. Amentoflavone isolated from methanol root extract is reported to have antidepressant and anxiolytic effect in mice [105]. Aqueous extract of the roots at doses of 13, 26 and 52kg/body weight restored to a high extent, sexual functions of paroxetine-induced sexual dysfunction in male Wistar rats. The extract also increased the levels of luteinizing and follicle stimulating hormones with a corresponding decrease in testosterone levels [109]. *Cnestis ferruginea* has been shown to have prevented gastric ulcer formation in immobilization stress induced gastric ulcer in mice [110]. Analgesic and anti-inflammatory property of the plant has also been studied. The extract (300-500mg/kg) produced a dose-dependant analgesic effect as well as inhibiting carrageenan-induced paw oedema and cotton pellet granuloma formation in rats [111]. Similar analgesic and anti-inflammatory activity has been reported Ishola et al., 2011, 2012 ([112], [105]). Fractions of the plant is reported to have considerable antioxidant activity using the free radical DPPH assay ([113], [101]).

Genotoxicity and Cytotoxicity Studies: *Cnestis ferruginea* leaves were implicated as the source of poisoning in Senegal some years back, where aqueous extract was found to be cytotoxic in a cell proliferation assay, XTT [114]. However,

in another study, methanol extract of the whole plant gave negative results in the bacterial reverse mutation assay. The extract did not cause any increase in His⁺ revertant colonies compared to the negative control even at a higher dose [115].

Paullinia pinnata

Description: *Paullinia pinnata* belongs to the family Sapindaceae. It is widespread in tropical Africa, tropical America and Madagascar. It is commonly called bread and cheese plant and locally known in Ghana as Akodwen (Fante), Toantini, Obosomfe bese (Twi), Gbadzafeka, Adfiehotsui (Ewe), Gbolantsere (Ga), and Yatsa-biya (Hausa). It is a woody climber with tendrils, up to 3cm high. The *leaves are imparipinnate with winged rachis. Inflorescences* axillary on long stalks, *bearing paired coiled tendrils*. It has white flowers and fruit about 2.5 cm long which is red when ripe [4].

Traditional Uses: The leaves are used for the treatment of gonorrhoea, dysentery, peptic ulcer, roots for cough, asthma, constipation, female infertility, haemorrhage, and the flowers for abdominal pains [4]. It is also used to treat diarrhoea, fracture, controlling blood pressure and as an aphrodisiac ([116], [117]).

Phytochemical Constituents: Presence of carbohydrates, reducing sugars, saponins, tannins, cardiac glycosides, anthracene derivatives have been reported Osarenmwinda et al., 2009 [118].

Antimicrobial Activity: Azelaic acid (a decarboxylic acid) from methanol root extract of *P. pinnata* has shown activity against both Gram negative and positive bacteria including resistant strains of *S. aureus* [117]. A lupeol-3-isovanniloyl ester isolated from the plant also demonstrated significant antibacterial activity against selected organisms including *Clostridium sporogenes* and anaerobic *C. tetani* [119]. The methanol extract is reported to exhibit a dose-dependent anti-diarrhoeal activity on whole mouse gastrointestinal tract [118]. Wound healing ability has been reported *in vitro* in human dermal fibroblast cell line 142BR and *in vivo* using excision and incision wound models in Sprague-Dawley rats. The methanol extract increased 142BR cell proliferation at 20µg/ml (94%), tensile strength, hydroxyproline content of healing tissue and also decreased epithelisation period and scar area [120].

Anti-parasitic Activity: Antimalarial activity of the ethanol leaf extract has been demonstrated in Swiss albino mice infected with *Plasmodium berghei*. The extract

suppressed parasitaemia in the early infective stages by 53 to 69% at 12.5, 25 and 50mg/kg per day. At the established stage, there was suppression of parasitaemia at 25 and 50mg/kg by 60%. [121]. Petroleum ether and methanol fractions exhibited larvicidal activity against *Anopheles gambiae*, with petroleum ether extract having the highest mortality rate of 100% at a dose range of 125 to 1000µg/ml after 24 hr exposure [122].

Pharmacological Studies: Biochemical and haematological indices of rats exposed to different doses of *P. pinnata* root extract showed a potential safety up to a dose of 850mg/kg [123]. Antioxidant activity of the methanol root and leaf extracts has been demonstrated by Zamble et al., 2006 [124]. In their study, the extracts were shown to induce a slight transcriptional activity of peroxisome proliferator activated receptor- α . The extracts also increased and decreased endothelial nitric oxide synthase and endothelin-1 mRNA levels in bovine aortic endothelial cells. Free radical scavenging activity on DPPH and 2, 2¹-azinobis-3-ethylbenzothiazoline-6-sulphonic acid radicals showed a strong scavenging activity [125]. Similar DPPH radical activity (IC₅₀ of 3.8µg/ml) with methanolic extract has also been reported by Annan et al., 2009 [117].

Genotoxicity and Cytotoxicity Studies: *Paullinia. pinnata* has been reported to have cytotoxic effects on HeLa, cervical carcinoma (25-50% cell proliferation), and HT29 colon adenocarcinoma (50-75% cell proliferation) cell lines at 10 and 100µg/ml [126]. *Paullinia. pinnata* methanol root extract exhibited moderate anticancer activity against human cell lines; DLD-1 and MCF-7 in MTT assay with IC₅₀ values 40-55µg/ml [127]. An antiproliferative activity by the plant has also been demonstrated in A431 squamous epidermal carcinoma, WM35 melanoma and A2780 ovary carcinoma cell lines [128].

***Vernonia amygdalina* (Del.)**

Description: *Vernonia amygdalina* belongs to the family Asteraceae and is commonly called bitter leaf. It is widespread in East and West Africa, but it is also grown in North Eastern South Africa and Yemen [129]. Commonly called bitter leaf and locally as Tatso (Ga), Biebingira (Dagbani), Gbo, Gboti (Ewe), Awonwone (Twi), Ayeawonle (Nzema). It is a perennial Shrub or small tree up to 10 m tall, much branched; trunk up to 40 cm in diameter; bark grey to brown, smooth, becoming fissured; young branches densely pubescent. Leaves alternate, simple;

stipules absent; Flowers bisexual, regular, 5-merous, strongly exserted from the involucre; Fruit a 10-ribbed achene 1.5–3.5 mm long, pubescent and glandular, brown to black, crowned by the much longer pappus bristles [130].

Traditional Uses: Bitter leaf is used in several dishes especially in West Africa. Leaf aqueous preparations are used to treat malaria, measles, dysentery, onchocerciasis, yellow fever, constipation and stomach pain [131]. The root and leaves are also used for kidney problems and hiccups ([97], [132]). In Ghana and Nigeria, the stem and root bark are used as chewing stick [133]. It is also used to treat oliguria, helminthiasis, pruritus, hypertension, cataract and loss of appetite [4].

Phytochemical Constituents: Phytochemical constituents found in the extract are tannins, alkaloids, saponins, glycosides, flavonoids, reducing sugars and coumarins [134].

Antimicrobial Activity: Antiviral activity of *V. amygdalina* fruit has been demonstrated in Poliovirus, where it reduced the viral titer by 10^3 [135]. Methanol leaf extract was found to be active against some bacterial isolates at 25mg/ml [136]. Crude and solvent fractions of the leaf and bark have also been found to be active against *E. coli* and *S. aureus* [137]. Similar antimicrobial activity of various fractions of the leaves was reported by Shama and Sharma, 2010 [138]. The plant extract showed a strong antibacterial activity against *S. mutans* and *S. aureus*. It was also active against multi-drug resistant gram-negative and gram-positive bacteria as well as against standard strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) [139]. Ibrahim et al., 2009 [134] also demonstrated the antimicrobial activity of aqueous and ethanol extracts on selected foodborne pathogens of medical significance including *E. coli*, *S. aureus*, *Bacillus cereus*, *Shigella dysenteriae* and *S. typhi*. Similar antibacterial activity has been reported in other literature ([140], [141], [142]), although earlier work by Otshudi, 1999 [143] on diarrhea causing pathogens did not show any significant activity. Two Sesquiterpene lactones, vernolide and vernodalol isolated from the leaves have been reported to possess both antibacterial (gram-positive bacteria) and antifungal activities. Vernolide had a higher activity with LC_{50} values of 0.2, 0.3 and 0.4 mg/ml against *Penicillium notatum*, *Aspergillus flavus*, *Aspergillus niger* and *Mucor hiemalis*. Vernodalol on the other hand, recorded LC_{50} values of 0.3, 0.4 and 0.5 mg/ml on the same fungal isolates [144].

Anti-parasitic Activity: Antiplasmodial activity of fractions from *V. amygdalina* leaves has been demonstrated against *Plasmodium falciparum* *in vitro*. Petroleum ether fraction showed a higher activity with $IC_{50} < 3\mu\text{g/ml}$ compared to that of ethanol fraction of $5 < IC_{50} < 10\mu\text{g/ml}$ [145]. The extract was also found to reduce gastrointestinal upset (flatulence and diarrhea) in wild chimpanzee infested with the parasite, *Ternidens* *sp.* There was reduction in parasitic infection from 1 hr. to 20.5 hr following ingestion of the extract [146]. However, the leaf extract did not exhibit any significant anthelmintic activity when tested against *Haemonchus cortortus* eggs *in vitro* at concentrations up to 11.2 mg/ml [147].

Pharmacological Studies: Antioxidant and cytoprotective activity of boiled, cold and methanol extracts of *V. amygdalina* has been reported to have DPPH free radical activity. (14.8 – 36.2%), it also gave a low haemagglutination titer of 0.32 to 5.56 in a haemagglutination assay [148]. Aqueous extract of the leaf administered up to 75% w/v or v/v to mature BALB/c mice did not show any clinical sign of toxicity [149]. An acute toxicity test of aqueous extract on liver function was shown not to be hepatotoxic; it had an LD_{50} of 500mg/kg [150]. Hepatoprotective activity has also been reported by Iwalokun et al., 2006 [151]. Pre-administration of the leaf extract to acetaminophen-induced hepatotoxicity and oxidative stress in mice *in vivo* resulted in a dose-dependent (50-100mg/kg) reversal of altered liver function parameters by 51.9 – 84.9%. Similar hepatoprotective activity was reported by Adesanoye and Farombi, 2010 [152]. In their work, the methanol leaf extracts reduced carbon tetrachloride-induced hepatotoxicity in male rats. Pre-administration of the extract at 250 and 500 mg/kg doses to the rats before CCl_4 (1.2 g/kg body weight) treatment resulted in reduction in concentration of serum hepatotoxicity indicator. The extract also increased activities of antioxidant enzymes; superoxide dismutase and glutathione S- transferase. It has also been reported to have an immunologic effect on HIV infected patients, therefore suggesting its use as adjuvant in HIV/AIDS management [153].

Genotoxicity and Cytotoxicity Studies: Antimutagenic activity of the organic solvent extracts of *V. amygdalina* leaves has been demonstrated by Obaseiki-Ebor et al., 1993 [154]. The extracts exhibited inhibitory effects on His^- to His^+ reverse mutation induced by ethyl methanesulfonate on *S. typhimurium* strain TA100 and that of 4-nitro-O-phenylenediamine and 2-aminofluorene on TA98. However, in a

cytotoxicity study of the aqueous extract of the plant using *Allium cepa* assay, [155] showed the extract was able to induce chromosomal aberration, which suggests the plant could cause genetic damage. Similar induction of cell aberration using *Allium cepa* assay has also been reported by Yekeen et al., 2011 [156]. *Vernonia amygdalina* has been reported to have anti-cancer effect on human breast cancer cells in an *in-vitro* MTT and Comet assays. Treatment of human breast adenocarcinoma cells (MCF-7) at different dose levels showed a significant ($P < 0.05$) reduction of MCF-7 cell viability, the comet assay also gave a slight dose-dependent increase in DNA damage in MCF-7 cells. There was minimal genotoxic damage in MCF-7 cells observed in the Comet assay [157]. The leaf extract has also been reported by Utoh and Anastasia, 2010 [158] to inhibit and destroy cancer cells during the early formative stages but did not effectively destroy the cells at advanced stages or prevent metastasis.

Momordica charantia

Description: *Momordica charantia* is a member of the family Cucurbitaceae. It grows in tropical areas such as East Africa, Asia, Caribbean and especially parts of the Amazon. It is also cultivated in South America. Commonly known as bitter melon, African or wild cucumber and bitter gourd. Other local names are Nyanya (Fante), Kakle (Ewe), Daddagu (Hausa) and Nyinya (Twi). The fruit resembles a young cucumber, appearing oblong and gourd. The young fruit is emerald green, but turns into orange-yellow when ripe. It splits into three irregular valves that curl backwards at maturity and releases numerous brown or white seeds encased in scarlet arils ([159], [160]). It has simple and alternate leaves with 3–7 deeply separated lobes” [161].

Traditional Uses: *M. charantia* or bitter lemon has long been used by the indigenous people in the Amazon. They grow it for food and medicine. The leaves are used to treat diabetes, hypertension, chicken pox, measles, wounds, fever and hepatitis ([160], [4]). The fruit extracts are used against stomach ulcer-causing bacteria, *Helicobacter pylori* and as a contraceptive. The root as astringent also used to treat leprosy, leucorrhoea, rheumatoid arthritis, lupus, pruritus, hypertension, gonorrhoea, and eczema, as a contraceptive and abortive, glucosuria, appetite stimulator, halitosis, haemorrhoids and gout among others [161].

Antimicrobial Activity: *M. charantia* seed is reported to have significant antibacterial activity against *S. aureus* at 125µg/ml (ATCC 6538) and 125-500µg/ml (clinical isolates). It also exhibited a time dependent killing activity after 8 hr exposure at 125µg/ml in a time kill assay [162]. The organic extracts of the seeds as well as the green parts were also sensitive against *Enterococcus faecium* with inhibition zones of 20-26mm [163]. The leaves have antifungal activity against *C. albicans*, *C. tropicalis* and *C. krusei* [164]. *M. charantia* extracts exhibited antiviral activity against Herpes simplex type I and II at < 5µg/ml [165] as well as antibacterial and antifungal activities ([166], [167], [168]).

Anti-parasitic Activity: Crude extract of the fresh fruit of the plant on IV instar mosquito larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* has been reported. Hexane extract exhibited a higher activity against the three mosquito species with LC₅₀ values of 66.05, 96.11 and 112.45ppm as compared to that of the crude extract at 0.50, 1.29 and 1.45% [169]. Anti-plasmodial activity of the aerial part of the plant against mice infected with *P. vinckei petteri* 279BY and *P. falciparum* chloroquine-resistant strain (Indo) has been investigated. The extract had moderate in vivo activity (70 % at 004mg/kg on *P. vinckei petteri*) and a high activity in vitro of 99% inhibition at 10mg/ml against *P. falciparum* [170]. Amorim et al., 1991 [171] earlier reported in their work that both aqueous and ethanolic extracts of the leaves were not effective at lowering parasitemia in *P. berghei* infected mice at concentrations of 500 -1000mg/kg [172] has also reported a weak anti-parasitic activity.

Pharmacological Studies: The anti-diabetic activity of *M. charantia* has been reported extensively in literature. The aqueous fruit extract reduced the blood glucose levels of rats administered with 50g in oral glucose tolerance test [173]. Fruit extracts administered to alloxan-induced diabetic rats over a 3 week period showed a significant (p<0.01) drop in blood sugar from 220mg% to 105mg%. Subsequent administration of powdered fruit and aqueous extract to severe to mild diabetic patients during a clinical trial also showed a significant drop in blood sugar levels, with the aqueous extract giving a high fall (54.0%) in blood sugar levels compared to the dried powder which gave a 25% fall in blood sugar level [174]. Similar study in alloxan-induced diabetic albino rats at a single dose of 250mg/kg of extract also showed a lowered blood glucose level over a 2 week trial period [175]. Saponin

fraction of the fruit extract administered to alloxan-induced hyperglycaemic and normal mice induced significant ($p < 0.05$), hypoglycaemic activity in both groups of mice [176]. Pure cucurbitanoid compounds isolated from *M. charantia* is also reported to exhibit *in vivo* hypoglycaemic effects on diabetic male ddY mice strain at a dose of 400mg/kg [177]. Reduced adiposity in rats fed on a hyperinsulinemic high fat diet (30%) supplemented with fruit extract at a dose of 0.75% or higher ($p < 0.05$) has also been reported by Chen et al., 2003 [178].

Genotoxicity and Cytotoxicity Studies: The seed extract was found to have decreased the genotoxic activity of methylnitrosamine, methanesulfonate and tetracycline [179]. The fruit extract has also been reported as having antioxidant and protective activities in male Wistar rats [180]. Research work by Sharma et al., 2011 [181] on the anti-carcinogenic effect of the aqueous extract of the fruit in mice, also showed extract reduced carcinogen-induced lipid peroxidation in the liver and DNA damage in lymphocyte. The extract was also reported to have inhibited tumour formation in the mice which had been injected with tumour cells.

***Strophanthus hispidus* DC**

Description: *Strophanthus hispidus* belongs to the family Apocynaceae and occurs naturally in savanna woodland and thicket. Arrow poison is the common name and it is also known locally as Omletswa, Anama (Ga), Amanfotama, maatwa (Twi), Ahoti (Ewe). It is a hairy climbing shrub. The leaves are simple, opposite, elliptic to ovate, 5-15cm long and 2-9cm broad. It has white flowers which turn yellow later [4].

Traditional Uses: The root and leaf are used to treat skin ulcers, leprosy, malaria, hypertension, venereal disease, yaws, arthritis and the stem for general body weakness [4]. Aqueous decoctions of the root and root bark are also used for rheumatic disease and oedema [182].

Pharmacological Constituents: Saponins, tannins, steroids, flavonoids, alkaloids, sapogenetic glycosides are the bioactive molecules present in this medicinal plant [183].

Antimicrobial Activity: Crude extract of *S. hispidus* is reported to inhibit the activity of *S. aureus* (NCTC 6571), *B. subtilis*, *E. coli* (NCTC 9001), *P. aeruginosa* (NCTC 6570), *A. niger* and *C. albicans* at concentrations of 10-100mg/ml [184].

Similarly, the methanol leaf and root extracts of the plant has also been reported by [183], to have antibacterial activity against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923) and *B. subtilis* with MIC values of 2.5-7.5mg/ml (leaves) and 2.5-10mg/ml (roots).

Pharmacological Studies: Treatment of carrageenan-induced hind paw oedema in rats, xylene induced ear oedema in mice and formalin-induced hind paw oedema in mice with the root extract has shown anti-inflammatory properties. The extract produced a dose-dependent inhibition of oedema in all groups of animals ($p < 0.05$). It also had significant inhibition on inflammatory mediators- histamine, serotonin and prostaglandin with the lowest inhibition of 42.8% being observed in serotonin [182]. The extract (7.5% w/v) applied topically over wounds 24hr for 24 days showed improved healing (Collagenation, re-epitheliasation and rapid granulation formation) in excision wound model female Sprague Dawley rats. The leaves and roots extract also showed low antioxidant activity in the DPPH assay with IC_{50} values of 49.8 and 45.1 μ g/ml respectively [183]. However, other DPPH assay done on the stem and root extract of *S. hispidus* did not give a positive outcomes [185].

Lippia multiflora

Description: *Lippia multiflora* belongs to the family Verbenaceae and generally found in tropical West Africa. Commonly called Gambia tea bush and also known locally as Afodoti, nyona (Ewe), Saresonunum, saanunum (Twi), Ngaa suru, Naa suru (Ga). A robust woody pubescent herby shrub up to 3 m high, bluish green leaves white or lilac fragrant flowers which are small and whitish with large oblong-lanceolate bluish-green leaves [97].

Traditional Uses: Aqueous leaf extract are used to treat gastrointestinal problems, high blood pressure, as a diuretic, muscle relaxant [186] and analgesic [187]. The roots are used to treat malaria and for placental retention [4].

Antimicrobial Activity: Leaf extract of *L. multiflora* and its isolated compound carvacrol exhibited activity on *Salmonella species*, *E. coli*, *S. aureus* and *B. subtilis*. *C. albicans* had the highest inhibition zone for both hexane extract (24mm) at 200mg/ml and carvacrol (38mm) at 4 μ l/disc [188]. The essential oil of the leaves had inhibitory effects on *S. aureus* (MIC 1.2 ± 0.1 mg/ml), *S. enterica* (MIC 4.4 ± 0.1 mg/ml) among others [189]. Reference [190] also reported some antimicrobial

activity of the essential oil. Whilst the methanol extract of the leaves had activity on *S. aureus*, *B. subtilis* and *E.coli*, it did not show any antiviral activity when tested on herpes simplex virus, sindbis and poliovirus. Similarly, the steam distilled volatile oil of the leaves also did not exhibit any antimicrobial activity (MIC 1250µg/ml) on the bacteria strains [191]. The leaves have been found earlier, to be active against isolated microorganisms of the buccal flora. Mouthwash prepared from the extract at 1/500 dilution of essential oil was also active [192].

Anti-parasitic Activity: *L. multiflora* leave extract administered at 400mg/kg dose to adult albino rats infected with *Trypanosoma brucei brucei* did not show any reduction in the level of parasitaemia [193].

Pharmacological Studies: The essential oil of the plant with monoterpenes as major constituent is reported to have analgesic and antipyretic activities. The effect of the oil (2, 4, 8 ml/kg) on acetic acid-induced writhings in Swiss mice (20-25g) showed a dose-dependent analgesic activity. The oil also exhibited antipyretic activity in brewer's yeast-induced hyperexic rats at the highest dose of 8 ml/kg; it however had no anti-inflammatory activity [187].

Genotoxicity and Cytotoxicity Studies: Continues exposure of Vero cells (Monkey kidney cell line) grown in Modified Eagle medium containing 5% foetal bovin serum to methanol leaf extract for 5 days showed cytotoxic effects at 125µg/ml [191]. Ashidi et al., 2010 [194] reported a moderate non-selective cytotoxicity of the plant on human breast adenocarcinoma cell line MCF-7, human large cell lung carcinoma cell line COR L23 and human amelanotic melanoma C32. The stem-bark of the plant has also been found to be also cytotoxic [195].

Khaya senegalensis

Description: *Khaya senegalensis* belongs to the family Meliaceae. It is a large tree that grows mainly in higher rainfall savannah woodlands and is native to Africa but can be found in Australia, Cuba and parts of Asia ([97], [116]). Commonly called mahogany, African mahogany other local names are Madwach (Hausa), Logo (Ewe), Okum (Fanti), Kuntunkuri (Twi). *K. senegalensis* is a deciduous evergreen tree up to 30(–35) m tall with a grey to dark grey or greyish brown bark surface. The leaves are arranged spirally but clustered near ends of branches. The flowers are unisexual, male and female flowers very similar in appearance, regular, and whitish [196].

Traditional Uses: *K. senegalensis* is used as a bitter tonic, as remedy for fever, vermifuge and for treating venereal diseases [41]. It is also used in treating malaria, as anthelmintic and mucus-containing diarrhoea [197]. Extracts of the stem-bark is also used for convulsion, hemorrhoids, heat rash, boils and arthritis [4].

Phytochemical Constituents: Saponins, tannins, phenols, alkaloids, cardiac glycosides and anthraquinones ([198], [199]).

Antimicrobial Activity: The methanol and aqueous extracts of *K. senegalensis* leaves have been reported to show antimicrobial activity against both gram-negative and positive-bacteria as well as *C. albicans* [199]. The ethanol extract of the stem-bark was highly active against *E.coli* and *S. typhi* at 20mg/ml [200]. Extracts of both leaf and bark also had some activity against *S. aureus* and *Enterobacter species* [201]. The root extracts has been reported to be active against *P. aeruginosa*, *B. subtilis*, *C. albicans* and *S. aureus* [202]. Limonoid compounds isolated from the leaves and twigs are reported to be active against MRSA and *P. aeruginosa* (MIC value of 12.5µg/ml) and antibacterial strains ([203] [204], [205], [198]). However, both aqueous and methanol extracts of the leaves did not show any activity when tested on bacterial isolates from post-surgical wounds [206]. The limonoid compounds of the plant had antifungal activity on *Botrytis cinerea*. It inhibited mycelia growth (60.83 to 68.33%) at 1000 to 1500ppm [207], the compound was also active against groundnut rust *Puccinia arachidis* [208].

Anti-parasitic Activity: Methanol extract from the bark of *K. senegalensis* had anti-parasitic activity (IC₅₀ 9.8µg/ml) when tested on *Leishmania donovani* [204]. The methanol extract at 4, 2 and 0.4mg/ml had trypanocidal activity on *T. brucei brucei* and *T. congolenses*, causing complete cessation of motility of the parasites within 60 minutes [209]. Various extracts of the plant tested for larvicidal activity on *Culex annulirostris* were active with LC₅₀ ranging between 5.1 to 20.12mg/ml [210]. Two compounds, Catechin-(4α, 6)-catechin and Catechin-(4α, 8)-catechin isolated from methanol extract of the plant were tested on *L. donovani*, *L. major* and *L. infantum* promastigotes and amastigotes. The compounds did not show activity against the promastigotes (EC₅₀ >50µg/ml), however, both were active on the amastigotes with EC₅₀ of 3.85 and 3.98µg/ml [211].

Pharmacological Studies: The effect of methanol bark extract of *K. senegalensis* on the cardiovascular system has been studied in rats. The extract at 1mg/ml was administered to urethane anesthetized rats with resting arterial blood pressure of 122±3mmHg, there was an increase ($p<0.001$) of blood pressure to 145.4mmHg [212]. The plant extract at concentration lower and above 1×10^{-5} g/ml induced a dose-dependent relaxation and contraction of the smooth muscle of rat bladder [213]. The stem-bark exhibited anti-inflammatory effects by inhibiting croton-induced mice ear oedema. At a dose of 150µg/ear, the chloroform extract inhibited the oedema by 70% compared with that of petroleum ether of 49% [214]. Administration of ethanol extract to rats at 2mg/kg body weight over a prolonged period of time (18 days) had a deleterious effect on the rat kidney [215]. The stem-bark, leaves and root have also been reported to have high antioxidant/free radical scavenging activity. The plant extracts exhibited radical scavenging potential with IC₅₀ values of 178µl (leaves), 91µl (stem-bark) and 122µl (root). The IC₅₀ values on the hypoxanthine/xanthine antioxidant assay for leaves, stem-bark and roots were 46, 37 and 64 respectively [216].

Cytotoxicity Studies: Catechin-(4α, 6)-Catechin and Catechin-(4α, 8)-Catechin tested on non-infective mammalian cell lines (RAW macrophages, squamous carcinoma (KB) and lung carcinoma A549) showed a non-toxic effect on the cell lines with effective doses (EC₅₀) of up to 25µg/ml [211]. The effect of leaves and bark extract of *K. senegalensis* on oxidative burst of isolated polymorphonuclear cells (PMNs) and mononuclear cells (MNCs) has been reported by Koko et al., 2008 [217]. Both extracts at 6.25µg/ml had some inhibitory effects (70.7, 67.1, 69.5 and 67.4%) on both phagocytes. Limonoid compound, 3α, 7α-dideacetylkhivorin from methanolic extract was found to have significant growth inhibitory (anticancer) activity on MCF-7, SiHa and Caco-2 cell lines with IC₅₀ of 0.07-0.14µM [218].

Kigelia Africana

Description: *Kigelia Africana* belongs to the family Bignoniaceae and is found across the African continent, with wide distribution in South, Central and West Africa. It spreads across riverside and wet savannah areas [219]. Commonly called sausage tree and known locally as Nufuten (Asante), Nyakpekpe (Ewe), Teeleo (Ga), Etua (Fante). It is a medium-sized, semi-deciduous tree which measures up to 25m

high. The bark is grey, smooth and flaky. Its leaves are opposite or whorled. The flowers are bisexual and very large with large sausage-like fruits [220].

Traditional Uses: The stem bark is also used to treat venereal diseases, epilepsy, wounds and oedema. The fruit and roots are used for the treatment of elephantiasis, snake bite, hemorrhoids, tapeworm infestation ([221], [4]).

Phytochemical Constituents: Presence of alkaloids, flavonoids, tannins, reducing sugars, saponins, glycosides and anthracene derivatives ([222], [223]).

Antimicrobial Activity: The ethanolic extract of the stem bark was active against *S. aureus* and *C. albicans* [222]. Antibacterial activity of hexane stem bark extract against *B. cereus* has also been reported [224]. Methanolic extract of the leaves and stem bark were active against *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus* and *C. albicans*, MIC ranged between 2.25 to 7.5mg/ml [182]. Other antimicrobial activity has been reported by Jeyachandran and Mahesh, 2007 [225].

Anti-parasitic activity: Leaf extract at 2mg/ml was active against nematode *Caenorhabditis elegans* and enteropathogenic *Entamoeba histolytica* [226]. The root and bark extracts of the plant exhibited a weak antitrypanosomal (IC_{50} -22 μ g/ml) and anti-plasmodial (IC_{50} > 5 μ g/ml) activity against *T. brucei rhodesiense* and *P. falciparum* [227].

Pharmacological Studies: Antidiarrheal activity of the aqueous extract on castor oil-induced diarrhea in adult male Wister rats has been reported by Akah, 1996 [223]. Results showed a significant ($p<0.01$) reduced fecal output. The extract also blocked ($p<0.01$) nicotine-induced contractions in the Guinea pig ileum activity test. Both leaf and stem-bark showed a low free radical scavenging activity (IC_{50} 13.7, stem-bark and 56.9 μ g/ml, leaves) in a DPPH assay. Wound treated with the extract showed significant healing from the 10th to 18th day [182]. Analgesic property of the plant has also been studied using acetic acid-induced mouse writhing as well as hot plate reaction time. In this study, there was a significant ($p<0.001$) inhibition of the writhes, however, the hot plate reaction time did not change. Extract of the plant is also reported to have reduced significantly ($p<0.05$), the production of thiobarbituric acid reactive substances (TBARS) induced by pro-oxidants in rat liver homogenate [228]. The leaf extract (100mg/kg) was again reported to possess hepatoprotective properties against acetaminophen-induced toxicity in mice [229]. The fruit is also

reported to have protective property against Cisplatin-induced toxicity in male rats [230].

Cytotoxicity and Genotoxicity Studies: The fruit extract of *K. Africana* tested negative for *Salmonella typhimurium* strains TA98 and TA100 in a mutagenicity test (without metabolic activation), while the same extract showed moderate cytotoxicity activity in a brine shrimp nauphlii bioassay [221]. The extract again gave negative results for strains TA98 and TA100 in a similar genotoxicity test by [231]. *Kigelia. africana* seed oil is also reported to have suppressed proliferation of Caca-2cell [232], and [219] reported n-hexane extract of two of its compounds were cytotoxic at higher concentrations.

***Mitragyna stipulosa*(DC.) Kuntze**

Description: *Mitragyna stipulosa* belongs to the family Rubiaceae. It is widely distributed across West Africa, in swamps and marshy localities. Commonly called African linden and locally known as Baya,-Subaha (Nzema), Supaya, Supaha-akoa (Asante). It is a medium size to fairly large tree of up to 35m tall. The bark is smooth to irregularly thin scaly or longitudinally fissured. The leaves are opposite, simple; stipules obovate to nearly round, up to 8 cm x 5 cm, hairy in lower parts. Flowers are bisexual, regular and scented. [233].

Traditional Uses: *Mitragyna stipulosa* is used mainly to treat malaria, diarrhoea, muscle pain, hypertension and for inflammation [234]. The stem-bark is used for female infertility treatment, skin ulcer and the leaves for wound healing [4].

Phytochemical constituents: The plant has been found to contain saponins and tanins [235].

Antimicrobial Activity: *Mitragyna stipulosa* bark extract exhibited antidiarrhoeal properties by showing activity against different strains of *E. coli* [235] and *Vibrio cholerae* [236].

Pharmacology and Cytotoxicity Studies: Two compounds, triterpenoids made up mainly of quinovic acid and glycoside derivative had inhibitory effects against snake venom phosphodiesterase with IC₅₀ values of 0.166± 0.013 and 0.374± 0.009mM respectively [237]. Triterpene derivative compounds isolated from *M. stipulosa* showed varying levels of cytotoxicity in an MTT assay with ursolic acid being the most active compound [238].

***Chlorophora excelsa* (Welw) Benth**

Description: *Chlorophora excelsa* belongs to the family *Moraceae*. It is native to Africa, widely distributed across tropical Africa and commonly called African teak or locally Odoum (Akan). It is a large tree which measures up to about 60m in height, with a dark colour with shades ranging from brownish yellow to green [239].

Traditional uses: Extracts from parts of the plant is used to treat cough associated with bronchitis, as an antibacterial, for lumbago, fever and rheumatism ([240], [41]). The stem bark is used as anti-inflammatory agent [241].

Phytochemical Constituents: Carbohydrate, tannins, glycosides, saponins, sterols, resins, flavonoids and alkaloids according to Abulude, 2007 [242].

Antimicrobial and Anti-parasitic Activity: Phenolic compounds, chlorophin and iroko isolated from aqueous extract of *C. excelsa* actively inhibited *B. coagulans* and *S. pneumoniae* at 1.95µg/ml (chlorophorin) and 3.125, 6.25µg/ml (iroko). The extracts were also active against fungal isolates *F. verticilloides* and *A. flavus*. Chlorophin and iroko also showed anti-parasitic activity against *E. histolytica* trophozoites with MIC of 0.25(chlorophin) and 1µg/ml (iroko) [243].

Pharmacological Studies: The stem-bark extract has been reported to have shown anti-inflammatory activity by significantly ($p < 0.05$) as shown by inhibition of carrageenan-induced rat paw oedema, in a cotton pellet granuloma formation test in rats [239]. It reduced cotton pellet dry weight ($p < 0.05$) at all concentrations and also inhibited ear oedema in mice at 400mg/kg after application of croton oil. Chlorophin has also been reported to possess antioxidant activity. The compound regulated unstabilised Hercules Ester gum 8D-LT and dipentene [244].

Genotoxicity and Cytotoxicity Studies: Chlorophorin was found to be non-mutagenic whilst *C. excelsa* was slightly mutagenic for the *Salmonella typhimurium* strain TA97. Both extracts showed no cytopathic effect on cell lines in an MTT assay [243].

Monodora myristica

Description: *Monodora myristica* belongs to the family *Annonaceae*. It is native to tropical West Africa, Uganda, Kenya and Tanzania. It is commonly called calabash nutmeg and locally known as Ayerew (Fante), Avoncoba (Nzema), Awerewa (Twi)

and Yikwi (Ewe). *M. myristica* is a branching tree of about 35m high. The leaves are large, about 35cm long and 18cm wide. The flowers are scented and waxy, with large woody fruits which are filled with brown seeds in a pulp ([94], [245]).

Traditional Uses: The seeds are used for anaemia, haemorrhoid, sexual weakness, wounds, numbness and dracunculiasis, the roots for arthritis [4]. It is also used for stomach ache, hypertension [246], malaria and constipation [247].

Phytochemical Constituents: Tannins, glycosides, flavonoids, steroids, cyanogenic glycosides ([248], [249]).

Antimicrobial Activity: The essential oil of the fruit had antibacterial activity on a range of microorganisms. It also had antifungal activity against *A. flavus* [250]. The fresh seed and essential oils also had activity against *B. subtilis*, *C. albicans* and *S. aureus* and other clinical isolates ([251], [248]). The dried seed essential oil was moderately active on mycotoxin producing fungi, *Fusarium moniliforme*, *A. flavus* and *A. fumigatus* at 500ppm [252]. Aderotimi, 2009 [253] reported a similar antifungal activity of the seed extract on *F. nivale*, *R. stolonifer* and *A. fumigatus*, fungi isolated from spoilt sweet potatoes. The ethyl acetate extract had a higher inhibition (9.5 ± 0.01 to 13.5 ± 0.1 mg/20ml) of fungal growth. Organic and aqueous seed extract at 200, 100 and 50mg/ml were active against selected bacterial strains and *C. albicans* with the organic extract showing a better activity (MIC 12.5 to 100mg/ml) on most strains [254]. Cyclohexane and ethyl acetate extract of fruit had good inhibitory effect on *C. albicans* and *C. krusei* (MIC 6.3 - 12.5 μ g/ml) ([255], [256]).

Anti-parasitic Activity: Crude extracts of *M. myristica* stem bark exhibited anti-helminthic activity (EC_{50} 2.5 μ g/ml) and anti-scabies activity (EC_{50} 2250mg/ml²) against nematode and mites. However, the extract was not active against *P. falciparum* and *T. brucei brucei* [257]. Both methanol and dichloromethane extracts of the plant were active against two chloroquine resistant strains of *P. falciparum* (FCB and W2). The methanol extract had a higher activity (IC_{50} 5.5 ± 2.0 and 6.1 ± 1.5 μ g/ml) as compared to that of dichloromethane (IC_{50} 4.3 and 28.1 ± 1.5 μ g/ml) against FCB and W2 strains of *P. falciparum* [247].

Pharmacological Studies: The essential oil of the leaves exhibited a weak anti-inflammatory effect on soybean lipoxygenase activity at 10 μ m ($28 \pm 0.7\%$) and a

weak antioxidant activity ($SC_{50} 15.0 \pm 0.8g/L$) in the DPPH assay [258]. Hypotensive effect of the seed extract has been reported by Koudou et al., 2007 [246]. At dose range of 40, 80 and $12\mu L kg^{-1}$, the oil reduced the blood pressure of guinea pig in a dose-dependent manner. The oil also caused a reduction in heart contraction of frog. The ethanol and aqueous extracts of the seed showed a strong antioxidant activity with the ethanol extract being more active than the aqueous extract. The extracts significantly ($p < 0.05$) caused reduction in DPPH radicals, reduced effect on Fe^{3+} and chelated Fe^{2+} . It was also protective against hydroxyl radicals and inhibited lipid peroxidation [259]. Similar antioxidant activity of the seeds has been reported by Uyoh et al., 2013 [260]. The extract had $37.37 \pm \%$ mean scavenging power and a hydroxyl radical scavenging power of $17.10 \pm 1.345\%$. It also had a reducing power of $0.074 \pm 0.001nm$ and a total antioxidant capacity (TAC) of $0.198 \pm 0.02nm$.

Cytotoxicity Studies: The methanol and dichloromethane extracts of the plant has been reported to exhibit cytotoxic effects on MRC-5 human diploid embryonic lung cell [247], it was also found to be cytotoxic on Human pancreatic cell lines [261].

Alchornia cordifolia

Description: *Alchornia cordifolia* belongs to the family Euphorbiaceae. It is distributed across Senegal east to Kenya and Tanzania, across Central Africa to West Africa. It is commonly called Christmas bush and locally known as Agyama (Twi), Gboo (Ga-Adamgbe), Avovlo(Ewe), Bambamie (Hausa). It is a straggling shrub or small tree of up to 8m tall. The leaves alternate, simple; stipules triangular and 1.5mm long. The flowers are unisexual, seeds ovoid, ellipsoid, smooth and bright red. The fruits are 2-lobbed capsule ([159], [262]).

Traditional Uses: The leaves are used for treating cough, bronchitis, malaria, headache, angina, dysentery and intestinal parasites [263] wounds, ringworm, yaws, abdominal pain, herpes zoster, dermatitis, jaundice, anaemia and dysmenorrhea. The roots are used for treatment of septicaemia, whooping cough and stomatitis [4]. It is also used for female infertility, peptic ulcer, as a purgative and for inflammation [262].

Phytochemical Constituents: Flavonoids, tannins, steroid, terpens, saponins and alkaloids [264].

Antimicrobial and Anti-parasitic Activity: Compounds isolated from methanolic leaf extract were active at 50mg/ml against *S. aureus* (16-28mm) and *E. coli* (12.5-19mm). Various fractions of isolated compounds at 100mg/ml were also active against *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa* [265]. In another study, the methanolic leaf extract was also active on *S. aureus* and *C. albicans* [266]. Aqueous extract of leaf extract tested against a wide range of clinical and environmental microorganisms at 5mg/ml inhibited 36.5% of isolates. The inhibition rate was 95.9% at 20mg/ml with most activity being against gram positive organisms and yeast [267]. Crude extract of the root bark is reported to exhibit anti-amoebic activity on clinically isolated *E. histolytica* strain (MIC of 62.5µg/ml) [264].

Pharmacological Studies: The ethanol leaf extract exhibited antidiarrheal activity on castor oil-induced diarrhea in mice. The extract in a dose dependent manner, reduced the frequency of diarrhea with 64.6% inhibition at 800mg/ml [268]. Fractions of leaf extract have been reported to have anti-inflammatory effect in egg albumin-induced rat hind paw oedema. The aqueous methanol extract showed a significant ($p<0.05$) 68.25% inhibition at 50mg/kg. Other fractions also showed activity at 100mg/ml with one fraction (A2) which had the highest activity of 50% inhibition also showing significant ($p<0.05$) analgesic activity [269]. Other anti-inflammatory activity of the methanol leaf extract has been reported by Manga et al., 2004 [262]. Fractions of the extract reduced croton oil-induced ear oedema in mice, with the hexane fraction having a higher inhibitory effect of 42% at 0.7µg/cm². The aqueous root extract at 100-400mg/kg significantly ($p<0.05$) inhibited writhing reflex, neurogenic and inflammatory pains in mice ([105].

In a toxicity study by Ansah et al., 2011 [270], the ethanol extract administered to rats at 250 and 500mg/kg did not exhibit any toxic effect. However, at higher doses (1000, 2000mg/kg), there were signs of hepatotoxicity in the rats. The seeds are also reported to be toxic when fed to broiler chicks [271]. The plant is reported to have showed antioxidant activity by reducing DPPH free radical and also had protective effect against carbon tetrachloride-induced liver damage in rats [272]. The effect of aqueous leaf extract on anxiolytic activity in mice using spontaneous movements and exploratory behavior was studied by Kamenan et al., 2013 [273]. The extract at 2500mg/kg showed a depressing effect on the central nervous system by reducing all spontaneous movement in mice. The plant at a minimum dose of 200mg/kg

significantly ($p < 0.05$) decreased the blood glucose levels of streptozotocin-induced diabetic rats, it also increased the levels of some haematological parameters (red cell count, haemoglobin concentration, total white cell count and lymphocytes) [274].

Genotoxicity and Sytotoxicity Studies: *Alchornea cordifolia* gave negative results in a genotoxicity study by [115]. The extract tested negative against the *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, and *Escherichia coli* WP2uvrA. Flavonoid-rich fractions from the plant have also been cited as having strong immunomodulatory activity [275], and the leaf extract has been reported to be non-cytotoxic ([276], [263]).

Terminalia ivorensis

Description: *Terminalia ivorensis* belongs to the family Combretaceae and is native to tropical West Africa, but can also be found in South America, Fiji and the Solomon Island [277]. It is common called black Afara and locally as Dzogbedodo (Ewe), Emire (Twi). It is a large deciduous forest tree between 15 to 46m tall. There are no branches up to 30m, and the mature trees are very flat topped with wide canopy horizontally. The branches of the young trees are whorled and the bark flakes off in long thin strips. The leaves are simple, whorled, oval and blunt tipped with orange-brown hairs below and on veins above ([277], [278]).

Traditional Uses: *Terminalia ivorensis* is used to treat arthritis, syphilis and as a diuretic ([279]. The stem bark is also used for wound healing and rheumatism [97].

Phytochemical Constituents: Saponins, tannins, flavonoids and alkaloids [280].

Antimicrobial and Anti-parasitic Activities: The aqueous and hydro-alcoholic bark extract had antifungal activity against *C. albicans* and *A. fumigatus*. The hydroalcoholic extract exhibited a high activity with minimal fungicidal concentration of 97.5µg/ml for both organisms and an IC_{50} of 11.40µg/ml (*C. albicans*) and 5.16µg/ml (*A. fumigatus*) [281]. The crude, chloroform and aqueous fractions were active against *S. aureus*, *E. coli* and *S. pneumonia* (MIC of 22.15 to 7937.04µg/ml) [282]. Ethyl acetate, ethenolic extract and their fractions exhibited antitrypanosomal activity against *T. brucei rhodensiense* [283]. Ethenolic stem bark extract of *T. ivorensis* at 25µg/ml showed an 83.34% inhibition against chloroquine resistant strains of *P. falciparum* with IC_{50} of 6.95µg/ml [280].

Pharmacological and Cytotoxicity Studies: Patients suffering from arthritis who were treated with bark extracts of *T. ivorensis* showed a remarkable improvement in their conditions and the extract at 100-300mg/kg also decreased carrageenan-induced rat paw oedema and delayed castor oil-induced diarrhea [284]. Compounds isolated from the bark (ivorensis B and C) had DPPH and ABTS radical scavenging activities. Ivorensis A exhibited anti-proliferative activity against MDA-MB-231 and HC7116 human cancer cell lines (IC₅₀ of 3.96 and 3.43μM) [285]. Crude, chloroform and aqueous extracts of the bark are also reported by Cobbinah, 2008 [282] to have antioxidant activity by exhibiting significant reducing power and decomposition of hydrogen peroxide.

Clausena anisata

Description: *Clausena anisata* belongs to the family Rutaceae. It occurs in Savanna, Riverine forest, and Secondary forest. It is distributed in tropical Africa, tropical Asia and South-East Asia. Commonly called horsewood or maggot killer. It is also known locally as Ayida, Ayra (Ewe), Samanyobli (Ga), Sesadua, Samanobere (Twi), Sesabaka (Nzema). *Clausena anisata* is a deciduous shrub or small tree of about 4m tall. The bark is smooth and leaves about 30cm long. The flowers are bisexual and irregular [286].

Traditional Uses: *C. anisata* is used for the treatment of fungal infection of the skin and oral candidiasis [287]. It is also used for epilepsy, anticonvulsant [288], for high blood pressure and as a mosquito repellent [289]. The roots and bark are also used for intestinal helminthiasis, dysentery, toothache, arthritis, herpes zoster, rheumatism, fracture and abdominal pains [4].

Phytochemical Constituents: Cardiac glycosides, tannins, saponins, terpenes and flavonoids [290].

Antimicrobial Activity: Two carbazole alkaloids, clausenol and clausenin isolated from the stem bark showed activity against both bacterial and fungal isolates [291]. *C. anisata* aqueous leaf extract showed moderate inhibitory effect on HIV-1 with EC₅₀ of 0.70mg/ml and 50% cytotoxic concentration (CC₅₀) of >1.4mg/ml [292]. The methanolic extract of the aerial parts of the plant at 1000-2000μg/ml had activity against *N. gonorrhoeae* [293], and the stem bark extract exhibited a strong antifungal activity against *C. neoformans*, *C. tropicalis*, *C. krusei* and *C. parapsilosis* [287].

The essential oil of the leaf extract, estragole-rich oil and trans-anethole-rich oil showed antimicrobial activity against both fungal and bacterial clinical isolates [294]. The essential oil of the leaves also showed activity against *S. typhimurium* and *P. aeruginosa* with MIC values of 62.5 and 125 µg/ml [295]. Similar antimicrobial activity of the essential oil has been reported by Guandidza et al., 1994 and Van Vuuren et al., 2006 [296] and [297].

Anti-parasitic Activity: The ethanolic and aqueous leaf extract of the plant showed anthelmintic activity inhibiting *Caenorhabditis elagans* [226]. The crude extract (39-1117mg/kg), chloroform and aqueous fractions (78mg/kg) of leaf extract significantly ($p < 0.001$) reduced the level of parasitaemia in mice infected with chloroquine resistant *P. berghei* [290].

Pharmacological Studies: Methanolic root extract at 100-800mg/kg administered to normal and streptozotocin induced diabetic rats produced significant ($p < 0.05-0.001$) dose-dependent hypoglycaemia [298]. The crude extract, chloroform and aqueous fractions significantly ($p < 0.001$) inhibited inflammation in mice by reducing acetic acid-induced writhing, formalin-induced hind paw licking and delayed hot plate pain reaction [290].

Genotoxicity and Cytotoxicity Studies: Imperatorin, isolated from *C. anisata* was found to be mutagenic using *Salmonella typhimurium* strains TA97, TA 98 and TA100 in Ames test [299]. However, alkaloid isolates from the plant have been reported to have antitumor promoting activity [300].

Piliostigma thonningii

Description: *Piliostigma thonningii* belongs to the family Fabaceae. It is found in wooded grassland and woodland in Sub-humid Africa and Asia. Commonly known as camel's foot or monkey bread, and locally called Opitipata, Tofotafa (Twi), Nyanga (Mole), Klo (Ewe). It is a single stem deciduous tree which measures about 4-15m long. The bark is rough with leathery green leaves of 15x17cm. The flowers are unisexual, but both male and female are usually on separate trees. Its pods which are indehiscent, measures up to 26x7cm and persist on the tree but finally fall and decay to pea-sized seeds ([301], [302]).

Traditional Uses: The roots and twigs are used to treat hookworm infestation, skin infections, dysentery, fever, respiratory ailments and snake bites [303]. Extracts from

the leaf are also used for malaria [304]. The leafy stem is used for pruritus, the stalk for chicken pox [4].

Phytochemical Constituents: Saponins, flavonoids, phenolics, glycosides, anthraquinones and cardiac glycosides [303].

Antimicrobial and Anti-parasitic Activity: Methanol extract of the stem bark at 20mg/ml had antibacterial activity against selected organisms (zone of inhibition of 10-17mm) including *S. pyogenes* and *S. dysenteriae* had a higher activity with MIC values of 0.313mg/ml for both organisms [305]. C-methylflavonol compounds isolated from the leaves were active against *S. aureus* whiles ethanolic extracts had activity against other selected bacteria strains [306]. *P. thonningii* exhibited toxic effects on L3 infected stage of *Haemonchus contortus*. It also had inhibitory effect on *Ascaris* (IC₅₀ 2, 10 and 15µg/ml) and *Onchocerca* (IC₅₀ 4, 8 and 28µg/ml). The extract at 100ppm also killed 50% of brine shrimp nauplii [307].

Pharmacological Studies: The aqueous and ethanol leaf extract of *P. thonningii* produced haemostatic effect on isolated aortic rat rings. The extract at 32mg/ml gave a better contractile response of 30.8%±1.6% of that produced by 1µM phenylephrine, whilst the ethanol extract at 64mg/ml produced 12.3%±0.9% contractile response. The aqueous extract also significantly (p<0.05) reduced bleeding time [308]. When administered to rats (200-400mg/kg) over 21 day period, it produced significant (p<0.05) decrease in haematological parameters (red blood cells, white blood cells, lymphocytes and neutrophils) and biochemical indices inferring that the plant might have possible effect on kidney function [309]. Aqueous extract at 0.2g/kg significantly (p<0.05) decreased the levels of serum lipids from 112.85±1.5 to 91.76±3.6mg/dl (Total cholesterol), 104.24±4.5 to 82.91±3.1mg/dl (Triglycerides) and 46.19±2.0 to 36.76±2.4mg/dl (LDL Cholesterol) [310].

The methanol leaf extract at 50, 100 and 200mg/kg, showed antiulcer effect *in vivo* in rats by reducing (57.1, 65.7 and 80% ulcer inhibition) indomethacin-induced gastric ulcer [311]. C-methylflavonols compounds isolated from the leaves, as well as crude and ethanol extracts have earlier been reported to have anti-inflammatory properties [307]. Ethyl acetate leaf extract exhibited hepatoprotective activity against toxicity induced by aluminium chloride [312]. Similar hepatoprotective activity of the leaves has been reported by [313]. In his study, the extracts at 50, 100 and

200mg/kg administered for 14 days to rats had protective effect acting as a free radical scavenger against carbon tetrachloride-induced hepatic and oxidative damage in the rats. A brine shrimp lethality assay also showed the plant was relatively non-toxic [314].

Trichilia monadelpha

Description: *Trichilia monadelpha* is a member of the family Meliaceae. It is found in lowland high forest, often on river banks in parts of Africa and is known locally as Otanduro (Twi), Tenuba (Nzema). It is a small to medium sized tree of about 12-20m high [97].

Traditional Uses: The aqueous roots are used for treating diarrhoea and dysentery, the bark and roots as depressants, for arthritis, rheumatism, yaws and as abortifacient. It is also used for subcutaneous parasitic infection, epilepsy and inflammation ([97], [116]).

Pharmacological Studies: Extracts of the stem bark of *T. monadelpha* exhibited antioxidant activity in a DPPH scavenging and reducing power test. Ethanolic and ethyl acetate extracts showed a stronger reducing activities (EC_{50} values of 0.87 ± 0.11 and 13.63 ± 0.38 mg/ml) compared to that of petroleum ether (EC_{50} 81.06 ± 4.35 mg/ml). All extracts had free radical scavenging activities [315]. The stem-bark extracts were also reported to reduce acetic acid-induced abdominal writhings in mice, increased paw withdrawal thresholds (maximum effect at 300mg/kg) in carrageenan-induced mechanical hyperalgesia test in rats and also significantly ($p < 0.0001$) inhibited neurogenic and inflammatory effects on right hind paw in mice with 5% formalin [316]. *Trichilia monadelpha* aqueous and petroleum ether extract inhibited carrageenan-induced foot-oedema (57.79 ± 3.92 and 63.83 ± 1.2) in chicks. The aqueous, petroleum ether as well as ethanol extracts also had inhibitory effects on inflammation associated with adjuvant-induced arthritis in rats [317]. Extracts of the plant are also reported to demonstrate excision wound healing properties and also showed dose dependent antimicrobial effects on intestinal microflora in colitic rats [318]. Aqueous root extract administered to male albino rats at 400mg/kg/day for 4 weeks had significant ($p < 0.01$) increase in sperm motility (from $5.75 \pm 0.38\%$ to $11.86 \pm 0.70\%$) and viability (from $13.3 \pm 1.16\%$ to $19.9 \pm 2.45\%$). It also showed a decrease in testosterone level [319].

Mussaenda erythrophylla

Description: *Mussaenda erythrophylla* belongs to the family Rubiaceae. It is native to Western tropical Africa and also seen as ornamental plant in India [320]. It is commonly called Ashanti blood or Red flag and known locally as Damarama, Akoko-ninidame (Asante), Akokonyindam (Fante). It is a climbing shrub of about 12m high with broad leaves, flowers are cream yellow or orange in appearance with an enlarges sepal (calyx) pink or red [4].

Traditional Uses: *Musseanda erythrophylla* is used traditionally for cough, diuretic, jaundice and as an appetizer [321]. It is also used to treat acute gastroenteritis, dysentery and laryngopharyngitis [322].

Phytochemical Constituents: Flavonoids, steroids, glycosides, triterpens, saponins and tannins ([321], [322]).

Anti-parasitic Activity: The ethyl acetate and methanol extract of the roots at 10, 20, 40 and 80mg/kg exhibited significant ($p < 0.01$) anthelmintic activity against *Pheretima posthuma* by causing paralysis and death of the worm. The ethyl acetate extract gave a shorter death time at 93 ± 8.40 mins compared to that of the methanol extract of 128 ± 5.22 mins [323].

Pharmacological Studies: The ethyl acetate and methanol stem extracts of the plant has been reported to be hepatoprotective in a carbon tetrachloride-induced hepatotoxicity test in rats. It has exhibited superoxide radical scavenging, lipid peroxidation inhibition, DPPH radical scavenging and hydroxyl radical scavenging activities [321]. Chloroform and ethanol extract are also reported to have diuretic effects in normal rats. The extracts, at 250 and 500mg/kg, increased urine volume, excretion of sodium and potassium. The extracts did not produce any toxic effects in mice when administered up to 2000mg/kg extract [322].

Anthocleista nobilis

Description: *Anthocleista nobilis* belongs to the family Loganiaceae, It is distributed in the tropical rainforest mainly in West Africa [324]. Cabbage tree or cabbage palm is the common name and the local name is Owudifo kete (Twi). It is small to medium –sized tree up to 18m tall. The bark is pale grey and smooth, leaves opposite and crowded at the end of branches. Flowers are bisexual and seeds obliquely ovoid-globuse [324].

Traditional Uses: Aqueous extracts of the plant are used traditionally for dysentery, fever, gonorrhea, diarrhea and stomachache [325], [326]). It is used for the treatment of diabetes mellitus, abdominal pains and malaria [129].

Antimicrobial and Anti-parasitic Activity: The methanolic extract had moderate antimicrobial activity against selected organisms. *Micrococcus flavus* had a higher activity (MIC value of 32µg/ml) compared to those of *B. subtilis*, *E. coli* and resistant strains of *S. aureus* (MIC values >512µg/ml) [327]. The ethanol extract of the root administered to *T. brucei brucei* infected mice did not reduce the level of parasitaemia [328]. The root extract at 0.5mg/100g is reported to have antiviral activity in Newcastle virus-infected fowls [329].

Pharmacological Studies: Ethanol root extract is reported to have a relaxation effect on guinea-pig ileum. The (67mg/kg) reduced pentobarbitone induced sleep time in chloroform poisoned mice. It also showed a dose-dependant toxic effect in mice with an LD₅₀ of 200mg/kg [328]. The plant extract showed a DPPH scavenging activity (IC₅₀ of 50.9µg/ml), 57% protection against hydrogen peroxide damage to fibroblast cells and also had *in vivo* wound healing effects on both excision and incision wound rat models [327]. The plant has earlier been reported by Duwiejua, 1983 [330] to have hypotensive activity.

***Desmodium adscendens* (Sw.) DC.**

Description: *Desmodium adscendens* belongs to the family Fabaceae. It is distributed across the Amazon rain forest of Peru, South America and West coast Africa [331]. It is locally known as Akwamfanu (Akan), Ananse, Nkatse (Fante), Azigbe, Anyigo (Ewe), Nkatenkate (Twi). It is a weedy perennial herb; multi branched and grows up to about 50cm tall. It produces numerous light purple flowers and small green fruits in 3cm long pods.

Traditional Uses: *Desmodium adscendens* is used for the management of Asthma, diseases with smooth muscle contraction [332], dysmenorrhea, wounds, infantile diarrhea [4], gonorrhea, excessive urination, ovarian inflammations, diarrhea, fever, pain and epilepsy [333].

Phytochemical Constituents: Phenols, Flavonoids, anthocyanins and tannins [334].

Anti-parasitic activity: The aqueous, ethanol and pentane extracts of the whole plant tested against two chloroquine-resistant *P. falciparum* strains; FCM29-

Cameroon and another strain from Nigeria showed a weak antiplasmodial activity. Of the three extracts, pentene had a better activity with IC₅₀ values of 41-70µg/ml (Nigerian resistant strain) and 35µg/ml (FCM 29 strain) [335].

Pharmacological Studies: *Desmodium adscendens* extracts and fractions are reported to have shown anaphylactic activity on guinea-pig. Both aqueous and ethanol extracts reduced anaphylactic contraction of guinea-pig ileum at various concentrations. The extract was observed to have reduced sensitivity of the ileum to histamine-induced spasms ([336], [337]). The leaf fractions are also reported to have effect on contractions of respiratory smooth muscle and contractions induced by histamine, carbachol and leukotriene D₄ [338]. Triterpenoid glycoside compounds isolated from the plant are reported to be potent activators of calcium-dependent potassium channels in smooth muscle [339]. Pre-treatment of mice with the extract (100, 300mg/kg) reduced pentylenetetrazole-induced (85mg/kg) convulsion and mortality. The extract also delayed significantly, the latency and limbic seizures in kainic acid (12mg/kg) induced seizures in rats. However, at 50, 100 and 300mg/kg, the extract had a low suppression of writhing induced by acetic acid by 26.05, 45.5 and 56.73% respectively [340]. The leaf extract at 25mg/ml is reported to reduce (83.21± 6.21%) reactive oxidative species generated by hydrogen peroxide. The extract also showed antioxidant activity in the ABTS (12.83mg) and DPPH (8.47mg) assays [336]. Reference [341] also reported similar antioxidant properties of flavonoid extracts of the plant. Another study on the antioxidant activity of the plant has been by [334].

Cytotoxicity Study: Extracts of the plant showed a weak cytotoxicity on A375 melanoma cells with pentane showing a better activity with IC₅₀ value of 70-90µg/ml [335]. Its immunoprotective property on both cellular and humoral immunity in mice has also been reported Rammal and Soulimani, 2011[342].

Conclusion

The role of medicinal plant products in the health care delivery system in Ghana and other developing countries cannot be over-emphasized. Various pharmacological, antimicrobial and cytotoxicity studies conducted by researches on these plants provide evidence and perhaps supports their use for various conditions claimed by

herbal medicine producers. However, more studies in relation to the longtime effects of using these medicinal products are needed to ascertain their safety.

Conflict of Interest

The authors declare no conflict of interest

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1.3 List of Commonly Marketed Ghanaian Medicinal plant Formulations targeted for Investigation in this Project

Table 2. Medicinal plant products with claimed therapeutic benefits

Product	Constituents	Claimed therapeutic benefits
E1	<i>Nauclea latifolia</i> , <i>Phyllanthus fraternus</i> , <i>Cryptolepis sanguinolenta</i>	Malaria, Typhoid fever
E2	<i>Ocimum gratissimum</i> , <i>N. latifolia</i> , <i>C. sanguinolenta</i>	Typhoid fever
E4	<i>Cnestis ferruginea</i> , <i>Paullinia pinnata</i> ,	Cough
E5	<i>Momordica charantia</i> <i>Vernonia amagdalina</i> , <i>Strophanthus hispidus</i> , <i>Lippia multiflora</i>	Diabetes
E6	<i>Khaya senegalensis</i> , <i>Kigelia africana</i> , <i>Mitragyna stipulosa</i>	Aneamia/tonic
E7	<i>Monodora myristica</i> , <i>Chlorophora excelsa</i> , <i>Alchornea cordifolia</i> , <i>K. senegalensis</i>	Haemorrhoids (piles)
E8	<i>Clausena anisata</i> , <i>Pilostigma thonningii</i> , <i>Trichilia monadelpha</i> , <i>K. senegalensis</i> , <i>N. latifolia</i>	Arthritis
E9	<i>Anthoscleista nobilis</i> , <i>Mussaenda erythrophylla</i> , <i>Vernonia amygdalina</i>	Candidiasis
E10	<i>Desmodium adscendens</i>	Asthma

1.4 Aims embodied in this MPhil Project

- ❖ To screen commonly marketed herbal medicinal products on the Ghanaian market for their mutagenic potential using the bacterial reverse mutation assay (Ames test)
- ❖ To determine the antimicrobial properties of commonly marketed Ghanaian medicinal plant product formulations.

2.1 *Salmonella typhimurium* Bacterial Reverse Mutation Assay (Ames test)

2.1.1 Bacterial strains

Salmonella typhimurium mutant strains (TA 98, TA 100, TA 1537) previously developed by Ames (1971), were obtained from the Microbiology Service Section, School of Biomedical Sciences, Curtin University. The *S. typhimurium* mutant strains were histidine dependent with either frameshift or base-pair substitution defects.

2.1.2 Cultivation and Storage of Bacterial Strains

The bacterial strains obtained were grown on Mueller Hinton (MH) agar by incubating the plates at 37°C overnight. Several colonies from the overnight cultures were each inoculated in vials containing plastic beads immersed in cryopreservative fluid (Thermo Fisher, Australia). The vials were inverted about ten times for emulsification of the colonies. The cryopreservative fluids were then removed before storage of the bacterial strains at -80°C freezer.

2.1.3 Validation of *Salmonella typhimurium* mutant strains

2.1.3.1 *rfa* mutation

The presence of *rfa* mutation is indicated by sensitivity to crystal violet (see section 1.2.2.2). About three colonies of *S. typhimurium* auxotrophs and a wild type strain were each inoculated in 6mL of labelled sterile nutrient broth (See Appendix A). The inoculated broths were incubated at 37°C overnight. Each strain was spread plated on MH agar and a disc containing 10µL of 0.1% crystal violet placed on the surface of the plates. The plates were inverted and incubated overnight at 37°C. A clear zone of inhibition observed around the crystal violet disc for *S. typhimurium* auxotroph, confirmed the presence of *rfa* mutation. The virulent strain however, was resistant to crystal violet.

2.1.3.2 R factor plasmid (pKM101)

The presence of pKM101 in *S. typhimurium* confers ampicillin resistance (see section 1.2.2.3). About three colonies of *S. typhimurium* auxotroph and wild type strain were each inoculated in 6mls of sterile nutrient agar broth. The broths were incubated at 37°C overnight and used for the spread plating on MH agar plates. A disc containing 10µg Ampicillin (Sigma-Aldrich, Australia) was placed on the surface of each plate. The plates were inverted and incubated overnight at 37°C. Ampicillin resistance of *Salmonella typhimurium* auxotroph was indicated by growth around the ampicillin disc. The *S. typhimurium* wild type strain had clear zone of inhibition around the disc using the CDS method (Bell, 1975).

2.1.3.3 Histidine and Biotin requirement

Four sets of glucose minimal (GM) agar plates (see Appendix A) were prepared for each *S. typhimurium* auxotroph and *S. typhimurium* virulent strain. For the first set of plates, 4 drops of sterile 5% Biotin solution was added to the middle of the plates. The solution was then spread on the entire surface of the plates using a sterile spreader. The second set of plates had 4 drops of sterile 5% Histidine and the third set contained a mixture of 5% Biotin and Histidine. The fourth plate did not contain any of these supplements. After overnight incubation, growth of *S. typhimurium* auxotroph was observed on the third plate only which was supplemented with 5% Biotin and Histidine, validating the mutants as histidine and biotin-dependent. The virulent strain on the other hand, had grown on all the four plates.

2.1.4 Medicinal plant products and Storage

The medicinal plant products used for the test were aqueous extracts obtained from the Ghanaian marketplace in Accra (Table 3). They are finished products packaged in bottles and sealed. The products were indicated for conditions such as malaria, typhoid fever, diabetes, piles, candidiasis, asthma, anaemia and arthritis. The plant extracts were freeze dried into powder form using a ScanVac CoolSafe freeze dryer (Fisherbiotic, Australia). The powdered products were labelled, sealed tightly and stored at 4°C until needed. A total number of nine products were freeze dried.

2.1.5 Determination of protein content of products

Estimation of the protein concentration of the medicinal plant products was determined by the Lowry *et al.* (1951) method using a 96-well microtitre plate method with bovine serum albumin (BSA) as the standard. The reagent used were 0.5mL of 1% copper (II) sulphate, 0.5mL of 2% sodium potassium tartrate and 50mL 2% sodium carbonate in 0.1N sodium hydroxide (biuret reagent) and 2N Folin-Ciocalteu's phenol reagent (see Appendix A).

Bovine Serum Albumin (BSA) was used to construct the standard curve for use in the quantification of the protein content of the commercial medicinal plant extracts. One milligram (1mg) of BSA was dissolved in 1mL of distilled water to obtain a final concentration of 1mg/mL protein. A serial dilution was prepared from the stock from 0, 1, 2.5, 5, 10 to a final protein concentration of 20µg/mL.

One hundred microliter (100µL) of each standard dilution was added to each well of a 96 well microtitre plate and the test samples were also added to their respective labelled wells. Two hundred microliter (200µL) of the Biuret reagent was added to each of the standard and sample wells, mixing upon each addition with repeated pipetting. The plate was then incubated at room temperature for 10-15 minutes. Twenty microliter (20µL) of the diluted Folin-Ciocalteu's phenol reagent was added to each well with repeated pipetting upon each addition. The plate was incubated for 30 minutes after which the absorbance was read at 650nm using Enspire multimode plate reader (PerkinElmer, Germany).

A standard curve was prepared from the absorbance readings of the standard solution. The concentration of the test samples were calculated from the standard curve. This was done by plotting the absorbance readings of the products on the standard curve and tracing it against the protein standard concentration.

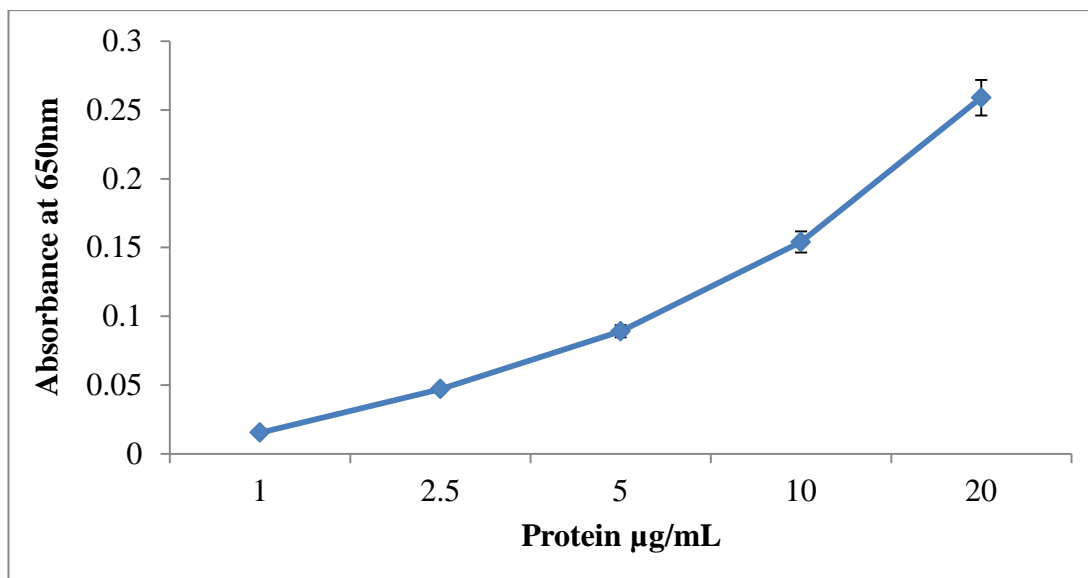


Figure 2: Protein content showing absorbance at 650nm (y-axis) against concentration in $\mu\text{g/mL}$ (x-axis). Values are represented as mean \pm SEM of three replicates

2.1.6 Determination of Carbohydrate (CHO) content of products

Carbohydrate content of the products was measured by the Phenol sulphuric acid method by Dubois et. al. (1956) using glucose as a standard.

Standard concentration of glucose was made in ten different concentrations ranging from 10-100 $\mu\text{g/mL}$. Dilutions of the test samples were prepared to a final volume of 0.5mL per test tube. One half of a millilitre (0.5mL) of 5% Phenol was added to each tube. The tubes were incubated at room temperature for 5 minutes. This was followed by the addition of 2.5mL of concentrated sulphuric acid (H_2SO_4), which was rapidly and directly added to the surface of the solution in a fume hood without touching the sides of the tubes. The tubes were mixed by vortexing and then placed in a water bath at 100°C for 10 minutes. The tubes were then allowed to cool to room temperature.

Absorbance was measured using a spectrophotometer at 490nm against an appropriate reagent blank which contained distilled water in the place of glucose. A standard curve was prepared from the absorbance readings and CHO concentrations of test samples determined from standard curve. This was done by plotting the absorbance readings of the products on the standard curve and tracing it against the CHO standard concentration.

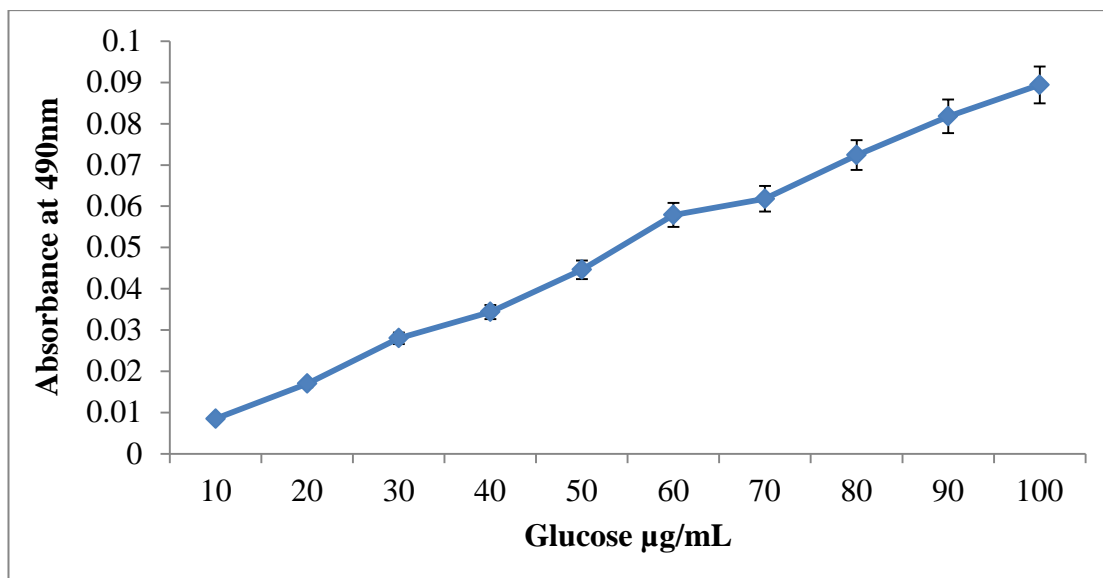


Figure 3: Carbohydrate content showing absorbance at 490nm (y-axis) against glucose concentration in µg/mL (x-axis). Values are represented as mean±SEM of three replicates

2.1.7 Preparation of Microsomal Fraction and Quantification of Cytochrome P450

2.1.7.1 Preparation of Microsomal Fraction from Pig liver

Fresh pig liver was obtained from the butchers' shop and the microsomal fraction was prepared according to the method described by Omura and Sato (1964) with slight modifications. The liver weighing about 65.5g was cut into small portions. The portions were divided to two groups. One litre of cold sodium chloride (0.9%) solution was added to one part of the liver portion (32.25g). For the second part, cytochrome P450 inducer, rifampin (ThermoFisher, Australia) was added to 1litre of cold sodium chloride (0.9%) solution at 3.275mg/L. Both samples were covered and allowed to perfuse overnight at 4°C.

The liver samples were then removed from the perfusion fluid and rapidly transferred into 1.15% ice-cold potassium chloride solution (Sigma, Australia). Each portion was finely sliced and gently homogenised in a sterile mortar and pestle with 4 volumes ice-cold 1.15% potassium chloride solution. The homogenate was centrifuged in a refrigerated centrifuge (Avanti J-E, Beckman coulter USA) at 20,000g for 25 minutes at 4°C. The floating fat layer was carefully removed and the supernatant microsomal fraction decanted. The precipitate was discarded. The microsomal fraction was again centrifuged at 100,000g for 90minutes at 4°C in an

ultracentrifuge (Optima XE-100 Ultracentrifuge, Beckman Coulter USA). The supernatant was discarded and the microsomal pellet resuspended in 4 times ice-cold 1.15% potassium chloride. The suspension was again centrifuged in the ultracentrifuge at 100,000g for 90 minutes at 4°C. The supernatant was discarded and the pellet resuspended in 1.15% of ice-cold potassium chloride solution at a concentration of 10mg/ml microsome. The samples were stored at -80°C. Protein content of the microsome was determined by the Lowry method (Lowry *et al*, 1951)

2.1.7.2 Quantification of Total Cytochrome P450 using the Ferrous CO versus Ferrous Difference Spectrum

2.1.7.2.1 Solubilization of Microsomal Fraction

The microsomal fraction was solubilized in a buffer which consisted of 100mM potassium phosphate buffer (pH 7.4) containing 0.1mM EDTA, 20% glycerol, 0.5% sodium cholate and 0.5% Triton X100 (non-ionic detergent).

2.1.7.2.2 Quantification of Total Cytochrome P450

Total P450 content of the microsomal fraction was measured using the Ferrous CO versus Ferrous difference Spectrum according to the method by Guengerich *et al* (2009) with slight modifications. Four hundred microliter (400µL) of the sample was diluted with 600µL of the solubilization buffer in two separate 1cm quartz cuvettes. About 1mg of sodium dithionite (the amount at the tip of a small spatula used for this purpose) was added to both cuvettes. The cuvettes were covered with parafilm and inverted without vigorous shaking to mix the contents. Both cuvettes were placed in a UV-2400 PC Series spectrophotometer (Shimadzu, China) and the baseline between 400 and 500nm was recorded. The sample cuvette was removed from the spectrophotometer and CO gas was slowly bubbled through the sample at the bottom of the cuvette for about thirty seconds at one bubble per second in a fume hood. The sample cuvette was placed back to its original position in the spectrophotometer and the absorbance was recorded between 400 and 500 nm for a several minutes.

The Cytochrome P450 concentration was calculated by the following formula:

$(\Delta A_{450} - \Delta A_{490}) / 0.091 = \text{nmol of P450/mL}$, where 0.091 is the Extinction Coefficient . The final concentration was expressed as P450 per mg protein (Guengerich et al, 2009).

2.1.8 Ames test Protocol

2.1.8.1 Test Principle

The test employs histidine-dependent *S. typhimurium* strains (*his*⁻ autotrophs), each carrying mutation in different genes in the histidine operon. When these autotrophs are grown on minimal agar plates containing a trace of histidine, only those strains that revert to histidine independence will grow showing visible colonies. Spontaneous mutation occurs with each strain, however, with the addition of a mutagen, the number of revertant colonies increases in a dose-dependant manner.

2.1.8.2 Tester Strain Media

M9 minimal agar plates used for the test was prepared from M9 salts, magnesium sulphate (1M), 20% glycerol, calcium chloride (1M) and 14g/L bacteriological agar (see Appendix A). The top agar for the overlay preparation was made from 6g/L bacteriological agar and 5g/L sodium chloride (see Appendix A).

2.1.8.3 S9 Fraction and S9 Mix

The S9 fraction was made from pig liver (see section 2.7). The S9 mix is a mixture of S9 microsome and co-factors (D-Glucose-6-phosphate, 1.6g/L, NADP, 3.5g/L, Magnesium sulphate, 1.8g/L, potassium sulphate, 2.7g/L, sodium phosphate dibasic, 12.8/L and sodium phosphate monobasic, 2.8g/L). (See Appendix A).

2.1.8.4 Dose Determination

Two dose levels of the products were prepared based on their protein and carbohydrate contents. The minimum dose based on the average the protein concentrations was 1mg/mL and the maximum dose based on highest carbohydrate concentrations of products was 8mg/mL.

2.1.8.5 Control and Standardization

All strains were tested with positive mutagenic chemicals to demonstrate the validity of the assay system and positive controls. The positive mutagenic chemicals were acridine orange (10µg/mL), sodium azide (1µg/mL) and neutral red (10µg/mL) for un-induced test and 2-aminoanthracene (10µg/mL) for S9 induced test. The negative control was sodium phosphate buffer, pH 7.4 (See Appendix A)

2.1.8.6 Experimental Procedure

The Ames test was performed using the pre-incubation method (Mortelmans and Zeiger, 2000). Two to three colonies of each bacterial strain was inoculated in 6mls of nutrient broth (Oxoid No 2). The broths were incubated at 37°C for 12-20 hr. Sterile test tubes were labelled for each test extract and controls. One hundred microliter (100µL) of each sample were added to their appropriate tubes followed by 100µL of the tester strains. Five hundred microliter (500µL) of phosphate buffer was added to the all the tubes for the non-metabolic step. In the metabolic activation system, 30% S9 Mix was prepared and kept on ice just before the test, which was added in place of the buffer. All tubes were mixed by vortexing and incubated for 20 minutes at 37°C. Pre-dried GM plates (90mm diameter) were labelled with the appropriate test samples. The top agar (18mL/bottle) was melted and supplemented with 2mL of 0.05mM histidine/biotin solution (see Appendix A) for each media bottle. The bottle was mixed by inverting. The top agar mixture (2.5mL) was added to each tube containing test samples (final volume of broth culture, 700µl), mixed and poured on the surface of the appropriately labelled GM agar plate allowing it to spread evenly on the plates. After the top agar had solidified, the plates were incubated for 48-72 hr at 37°C. All test were done in triplicates. The revertant colonies were counted using colony counter (Gallenkamp, England) and the results expressed as mean number of revertants per plate for each test sample.

2.2 Antimicrobial Activity of Medicinal Plant Products

2.2.1 Medicinal Products

The nine medicinal extracts used for the antimicrobial activity testing were aqueous medicinal products from the Ghanaian marketplace. These were processed and stored until needed as shown in section 2.1.4.

2.2.2 Microorganisms

The microorganisms used for the antimicrobial test consisted of *Klebsiella pneumoniae*, *Streptococcus agalactiae*, *Acinetobacter baumannii*, *Moraxella catarrhalis*, *Streptococcus pyogenes*, *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Bordetella parapertussis*, Methicillin-resistant and Methicillin-sensitive *Staphylococcus aureus* (MRSA and MSSA). These organisms were clinical isolates obtained from the Microbiology Service Section, School of Biomedical Sciences, Curtin University through the help of Mr. Alain Delhaize.

2.2.3 Cultivation and Storage of Microorganisms

A single colony of *C. albicans*, *K. pneumoniae*, *S. typhimurium*, *E. coli*, *P.aeruginosa*, MRSA and MSSA obtained was inoculated onto their respective labelled Muller Hinton (MH) plates. The plates were incubated at 37°C for 24hr. *S. pyogenes*, *S. agalactiae*, *A. baumannii*, *M. catarrhalis* and *B. parapertussis* were inoculated onto blood agar plates and incubated at 37°C for 24hr except *B. parapertussis* which was incubated in an enclosed container with moist tissue and incubated for 48hr. Several colonies from the overnight cultures were each inoculated in duplicates into vials containing plastic beads immersed in cryopreservative fluid (Thermo Fisher, Australia). The vials were inverted about ten times for emulsification of the colonies. The cryopreservative fluids were then removed before storage of the isolates at -80°C freezer.

2.2.4 Agar diffusion test

Antimicrobial activity of the extracts was performed by the agar-well diffusion method as described by Magaldi *et al* (2004). Fresh cultures of the isolates were made on MH and blood agar. 2-3 colonies of the microbial isolates were inoculated into 3.5mL of sterile saline solution. These were incubated overnight at 37°C. After the overnight incubation, turbidity of the suspensions were adjusted to 0.5 McFarland standards (1.5×10^8 cfu/mL). A lawn inoculum of each isolate was made on either MH (*C. albicans*, *K. pneumoniae*, *S. typhimurium*, *E. coli*, *P.aeruginosa*, MRSA and MSSA) and blood agar (*S. pyogenes*, *S. agalactiae*, *A. baumannii*, *M. catarrhalis* and *B. parapertussis*) plates using sterile cotton swabs. The plates were labelled and a

6mm well was made in each plate using a sterile cork borer of 6mm diameter. One hundred microlitre (100µL) of each extract (2mg/mL) was added to their respective labelled wells. The plates were then refrigerated at 4°C for 2hr to allow diffusion of extracts to the culture medium. After the diffusion of extracts, the plates were incubated at 37°C for up to 24hr except *B. paraptussis* which was incubated for 48hr in a moist enclosed container. The zones of inhibition were measured using a measuring rule and all test were done in triplicates. The antibiogram of the isolates were also determined to ascertain the resistance/susceptibility of isolates to antibiotics. This was done using the calibrated dichotomus sensitivity test, CDS-method by Bell (1975). The CDS disc method of antibiotic sensitivity testing (calibrated dichotomous sensitivity test). Pathology, 7(4 Suppl): Suppl 1-48.

2.2.5 Determination of MIC of the Commercial Ghanaian Medicinal Plant Products

The minimum inhibitory concentration (MIC) of product with antimicrobial activity was determined using the 96-well microdilution method (Koneman, 2006, CLSI, 2012). In this test, a stock solution of 20mg/mL of product was prepared. Two hundred microliter (200µl) of extract was added to the first well of the microtitre plate. A two-fold dilution of the extract was made from the first well by serially diluting 100µL of the extract in 100µL of Brain Heart infusion broth from the second well to the eleventh well, giving a concentration of 2mg in the first well to 0.002mg in the eleventh well (2000µg to 1.953µg). The twelfth well was left as the solvent control. In this test, sterile distilled water was used as solvent control for the extracts and DMSO as solvent control for antibiotics used. Trimethoprim and Cephalexin were used as standard antibiotic controls. These were also serially diluted as the extracts from 500µg (first well) to 0.488µg (eleventh well). The plates were incubated at 37°C for 24 hr. All tests were done in triplicate. After overnight incubation at 37°C, 40µL of 0.2mg/ml p-iodonitrotetrazoliumviolet (INT) was added to all the wells and the plates examined within 30 minutes for bacterial growth. Bacteria growth was indicated by the red colour of INT reducing to formazan. The lowest concentration at which decrease in the red colour was apparent compared to the next dilution was taken as the MIC value. To determine the minimum inhibitory concentration (MBC), all wells with no visible growth were inoculated on agar plates and incubated overnight at 37°C. Plates were observed for growth after incubation,

the lowest concentration at which there was no growth on the agar plate was considered the MBC value.

**Mutagenic Potential of Commonly Marketed Ghanaian
Medicinal Plant Products**

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Mutagenic Potential of Commonly Marketed Ghanaian Medicinal Plant Formulations using Bacterial Reverse Mutation assay

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Abstract

The mutagenic potential of medicinal plants commonly sold on the Ghanaian market was evaluated in this study. The plant formulations were aqueous extracts prepared by the manufacturers from a combination of two or more plants. The Mutagenicity tests were conducted on a total of nine herbal formulations using *Salmonella*/microsome bacterial reverse mutation assay (Ames test) with or without metabolic activation. The *Salmonella typhimurium* mutant strains used for the test were TA98, TA100 and TA1537. None of the products tested indicated a positive mutagenic effect on the mutant *Salmonellae* strains. One of the extracts, E8 containing a combination of aqueous extracts from *Clausena anisata*, *Pilostigma thonningii*, *Trichilia monadelpha*, *Khaya senegalensis* and *Nauclea latifolia* showed a 2-fold decrease in revertant colonies compared to that of the negative controls for

all strains in the metabolic activation assay, indicating the potential antimutagenic activity of extract.

Key words: Mutagenicity, Medicinal plants, *Salmonella typhimurium*, Ames test, Ghanaian marketplace

Introduction

Medicinal plants have been in use since time immemorial by traditional healers for the treatment and management of many health conditions. Medicinal plants have also been found to contain bioactive compounds in many plants extracts which accounts for their medicinal properties [1]. Based on their long-term use without sufficient scientific data on their safety or toxicity, it is generally believed that herbal medicines are safe or have low toxicity. However, recent reports on the adverse reactions to herbal medicine ([2], [3], [4], [5], [6]) and mutagenic potential of some plant extract ([7], [8], [9], [10]) raises concern over the long-term use of some medicinal plants.

The aim of this study was to screen commonly marketed herbal medicinal products on the Ghanaian market for their mutagenic potential using the bacterial reverse mutation assay (Ames test). This test is a correlate of compounds that may cause cancer. The ideal method to assess the carcinogenic potential of products including foods, therapeutic drugs including medicinal plants is to test their ability to form tumors in animals, an expensive proposition.

A brief description of the medicinal plants used in different extracts is as follows:

Nauclea latifolia (Sm): This plant, a member of the Rubiaceae family, is a multi-stemmed shrub or small tree which is also cultivated worldwide. It is commonly called African peach or pin cushion tree, used traditionally for the treatment of diarrhoea, malaria, hepatitis and stomach disorders ([11], [12], [13]).

Phyllanthus fraternus (Webster): This medicinal plant, a member of the Euphorbiaceae, is native to the Amazon rain forest and India. It is glabrous herb used in the treatment of intestinal disorders, viral infections, jaundice, dysentery, stomach ache and the management of diabetes ([14], [15], [11]).

***Cryptolepis sanguinolenta*:** *Cryptolepis sanguinolenta* belongs to the family Periplocaceae and commonly found in West Africa. It is a shrub used traditionally in the treatment of malaria, urinary tract infections, upper respiratory infections, hypertension and inflammatory disease ([16], [17]).

***Ocimum gratissimum*:** *Ocimum gratissimum* is a member of the Lamiaceae (Labiatae) which is native to Africa, Western India and Asia. It is an aromatic herb commonly known as basil or tea bush. It is traditionally used in the treatment of skin diseases, cough, upper respiratory infections, malaria, snake bites, diarrhoea and dysentery ([18], [11]).

***Cnestis ferruginea* (Vahl) D.C:** This medicinal plant which is commonly found in tropical regions especially West Africa, is a member of the family Connaraceae. It is a shrub used in the treatment of urethral discharge, gynaecological conditions, skin and wound infections, headache and sinusitis ([19], [20], [11]).

***Paullinia pinnata*:** *Paullinia pinnata* is a member of Sapindaceae family, common in tropical Africa, America and Madagascar. It is commonly called bread or cheese plant. It is used for the treatment of peptic ulcer, female infertility, asthma, and cough. It is also used to control blood pressure and as an aphrodisiac ([21], [22], [11]).

***Vernonia amygdalina* (Del.):** *Vernonia amygdalina* is commonly called bitter leaf and belongs to the family Asteraceae. It is a perennial shrub which is widespread over Africa. Aside its use as food and chewing stick, it is also used in the treatment of conditions such as kidney diseases, measles, malaria, cataract, onchocerciasis, yellow fever and helminthiasis ([23], [24], [25], [11]).

***Momordica charantia*:** *Momordica charantia* is a tropical plant commonly found in Africa, Asia and parts of the Amazon. It is a member of the Cucurbitaceae family commonly called bitter melon, African or wild cucumber or bitter gourd. Aside from its use as food, *M. charantia* is also used in the treatment of hypertension, chicken pox, hepatitis, rheumatoid arthritis, lupus, glucosuria and as a contraceptive ([26], [11] [27]).

***Strophanthus hispidus* (DC):** *Strophanthus hispidus*, also called arrow poison. A hairy climbing shrub found in savannah woodlands and a member of the Apocynaceae family. Extracts of the plant are used in the treatment of venereal diseases, arthritis, rheumatic disease and oedema ([28], [11]).

***Lippia multiflora*:** *Lippia multiflora* is a member of the Verbenaceae family and is native to tropical West Africa. It is a herby shrub commonly called Gambia tea bush. *L. multiflora* is used in the treatment of gastrointestinal infections, high blood pressure, analgesic and a muscle relaxant ([19], [11], [29]).

***Khaya senegalensis*:** *Khaya senegalensis* is a large tree belonging to the Meliaceae family. It is native to Africa and commonly called African Mahogany. *K. senegalensis* is traditionally used as a bitter tonic, for the treatment of malaria, boils, arthritis and as anthelmintic ([21], [19], [17], [11]).

***Kigelia africana*:** *Kigelia africana* belongs to the family Bignoniaceae and is widely distributed across Africa. It is commonly called sausage tree and used traditionally for the management of epilepsy, elephantiasis, worm infestations and haemorrhoids ([30], [31]).

***Mitragyna stipulosa* (DC.) Kuntze:** Commonly called African linden, *M. stipulosa* is a large tree belonging to the Rubiaceae family and native to West Africa. It is used in the treatment of inflammatory conditions, diarrhoea, female infertility and skin ulcer ([11], [32], [33]).

***Chlorophora excelsa* (Welw) Benth:** *Chlorophora excelsa* is a member of the Moraceae family and is native to Africa. It is a large tree commonly called African teak, and is used traditionally in the treatment of conditions such as rheumatism and cough associated with bronchitis. It is also used as antibacterial and anti-inflammatory agent ([34], [35]).

***Monodora myristica*:** *Monodora myristica* belongs to the family Annonaceae, native to Africa and commonly called calabash nutmeg. It is a branching tree used in the

treatment of anaemia, sexual weakness, hypertension, malaria and stomach ache ([36], [19], [37], [38]).

Alchonia cordifolia: *Alchonia cordifolia* belongs to the Euphorbiaceae family and also known as Christmas bush. It is widely distributed across Africa, and is used for treating intestinal parasites, dermatitis, herpes zoster, ringworm, peptic ulcer and whooping cough ([39], [40], [41]).

Terminalia ivorensis: This medicinal plant is a member of the Combretaceae family and native to West Africa. It is a large tree commonly called black afara. It is used traditionally for wound healing and rheumatism ([19], [42], [43]).

Clausena anisata: *Clausena anisata* is a deciduous shrub or small tree belonging to the Rutaceae family and commonly called horsewood or maggot killer. It is usually found in savannah, riverine and secondary forest. *C. anisata* is used for treating fungal infections of both skin and mouth. It is also used in the management of high blood pressure, epilepsy and as a mosquito repellent ([44], [45]).

Piliostigma thonningii: Also known as camel's foot, *P. thonningii* is a member of the Fabaceae family and commonly found in Sub-humid Africa and Asia. It is used in the treatment of hookworm infections, respiratory diseases, dysentery and snake bites ([46], [47]).

Trichilia monadelpha: *Trichilia monadelpha* belongs to the Meliceae family and is commonly found on river banks in Africa. It is used in the treatment of rheumatism, diarrhoea, yaws, inflammation, arthritis and as an abortifacient ([21], [19]).

Mussaenda erythrophylla: *Mussaenda erythrophylla* is a climbing shrub belonging to the Rubiaceae family. It is native to tropical Africa and India and is commonly called Ashanti blood or red flag. It is used for treating cough, jaundice and as a diuretic ([11], [48]).

Anthoscleista nobilis: *Anthoscleista nobilis* belongs to the Loganiaceae family and commonly called cabbage tree or cabbage palm. It is usually found in tropical

rainforest of West Africa and used traditionally in the treatment of dysentery, diabetes mellitus, abdominal pains, diarrhoea and abdominal pains ([49], [50], [51], [52]).

Desmodium adscendens (Sw.) DC: *Desmodium adscendens* is a weedy perennial herb belonging to the Fabaceae family. It is found across the Amazon forest, West Africa and South America. It is used in the treatment of infantile diarrhoea, excessive urination, gonorrhoea, asthma and epilepsy. ([53], [54], [11]).

Materials And Methods

Medicinal Plant Products

Medicinal plant products used in this study were purchased from the Ghanaian marketplace already prepared by the various herbal manufacturers and consisted of combinations of aqueous extracts (Table 1). The products were indicated for a variety of diseases such as malaria, typhoid fever, diabetes, piles, candidiasis, asthma, anaemia and arthritis. The herbal products were freeze dried into powder forms using a ScanVac coolsafe freeze dryer (Fisherbiotic, Australia) and reconstituted into 1mg and 8mg /mL concentrations according to their protein or carbohydrate concentrations. Extracts were stored in the refrigerator at 4°C until needed.

Table 1 Medicinal products (Extracts) and their plant constituents

Extract	Plant Constituents
E1	<i>Nauclea latifolia, Phyllanthus fraternus, Cryptolepis sanguinolenta</i>
E2	<i>Ocimum gratissimum, N. latifolia, C. sanguinolenta</i>
E4	<i>Cnestis ferruginea, Paullinia pinnata</i>
E5	<i>Momordica charantia Vernonia amagdalina, Strophanthus hispidus, Lippia multiflora</i>
E6	<i>Khaya senegalensis, Kigelia africana, Mitragyna stipulosa</i>
E7	<i>Monodora myristica, Chlorophora excelsa, Alchornea cordifolia, K. senegalensis</i>
E8	<i>Clausena anisata, Pilostigma thonningii, Trichilia monadelpha, K. senegalensis, N. latifolia</i>
E9	<i>Anthoscleista nobilis, Mussaenda erythrophylla, Vernonia amygdalina</i>
E10	<i>Desmodium adscendens</i>

E=extract

Bacterial Strains

Salmonella typhimurium mutant strains (TA98, TA100, TA1537) previously developed by reference [55], were obtained from the Microbiology Service Section, School of Biomedical Sciences, Curtin University. The *S. typhimurium* mutant strains were histidine-dependent with either frameshift or base-pair substitution defects.

Table 2 Characteristics of *Salmonella typhimurium* mutant strains used*

<i>Salmonella</i> Strain	Affected Gene	DNA Repair	LPS	Biotin Requirement	R Factor Plasmid	Mutational Event
TA98	hisD3052	uvrB	rfa	bio-	pKM101	Frameshift
TA100	hisG46	uvrB	rfa	bio-	pKM101	Base-pair substitution
TA1537	hisC3076	uvrB	rfa	bio-	-	Frameshift

*Described in Ames BN, Lee FD, Durston WE. (1973). "An improved bacterial test system for the detection and classification of mutagens and carcinogens". *Proc Natl Acad Sci, USA*. 70:782-786

Experimental Design

Bacterial Reverse Mutation Assay (Ames test)

Glucose minimal (GM) agar plates of 90mm diameter were prepared using bacteriological agar (14g/L), M9 salts, 5x (Sigma Aldrich), 20% glycerol, Magnesium sulphate (2mL of 1M solution) and calcium chloride (100µL of 1M solution). Top agar was prepared using bacteriological agar (6g/L) and sodium chloride (5g/L). Histidine/Biotin solution was 0.05mM. S9 Mix was prepared prior to the start of test from S9 fraction and co-factors solution containing glucose-6-phosphate (Sigma-aldrich, Australia), nicotinamide adenine dinucleotide phosphate (Roche, Australia), magnesium chloride, potassium chloride, sodium phosphate monobasic and dibasic (Sigma-aldrich, Australia).

The Ames test was performed by the pre-incubation method according to the method described by Mortelmans and Zeiger, 2000 [56]. Two to three colonies of each bacterial strain were inoculated in 6mls of nutrient broth (Oxoid No 2). The broths were incubated at 37°C for 12-20 hr. One hundred microliter (100µL) of each sample was added to their appropriate tubes followed by 100µL of the tester strains. In the non-metabolic activation test, 500µL of sodium phosphate buffer (pH 7.4) was added to sample tubes and control whilst the metabolic activation system had 500µL of 30% S9 Mix added in place of the buffer. All tubes were mixed by vortexing and incubated for 20 minutes at 37°C. Two millilitres (2mL) of histidine/biotin solution was added to 18mL of molten top agar and the content mixed by inverting. Two and a half millilitres (2.5mL) of the top agar mixture was added to each tube containing test samples (total broth culture of 700µl), mixing after each addition and immediately pouring content onto the surface of the appropriate labelled GM agar plate allowing it to spread evenly on the plates. The plates were allowed to set for thirty minutes before incubation at 37°C for 48-72 hr. All tests were done in triplicates. A lawn of bacteria growth was observed with revertant colonies appearing on the surface. The revertant colonies were counted using colony counter (Gallenkamp, England) and the results expressed as mean number of revertants per plate for each test sample.

Preparation of Microsomal Fraction from Adult Pig liver

Fresh pig liver was obtained from the butchers' shop and the microsomal fraction was prepared according to the method described by Omura and Sato, 1964 [57]. The liver was cut into small portions and divided into two. 1 litre of cold sodium chloride (0.9%) solution only was added the first portion. The second portion had cytochrome P450 inducer, rifampin (ThermoFisher, Australia) added to 1litre of cold sodium chloride (0.9%) solution at 3.275mg/L concentration. Both samples were covered and allowed to perfuse overnight at 4°C. After the overnight perfusion, liver samples were removed from the perfusion fluid and rapidly transferred into 1.15% ice-cold potassium chloride solution (Sigma, Australia). Each portion was finely sliced and gently homogenised using a sterile mortar and pestle with 4 volumes ice-cold 1.15% potassium chloride solution. The homogenates were centrifuged in a refrigerated centrifuge (Avanti J-E, Beckman coulter USA) at 20,000g for 25 minutes at 4°C. The floating fat layer was carefully removed and the supernatant microsomal fraction decanted. The microsomal fraction was centrifuged at 100,000g for 90minutes at 4°C in an ultracentrifuge (Optima XE-100 Ultracentrifuge, Beckman Coulter USA). The supernatant was discarded and the microsomal pellet resuspended in 4 volumes ice-cold 1.15% potassium chloride. The suspension was again centrifuged in the ultracentrifuge at 100,000g for 90 minutes at 4°C. The microsomal pellet was resuspended in 1.15% of ice-cold potassium chloride solution at a concentration of 10mg/ml microsome. The samples were stored at -80°C. Protein content of the microsome was determined by Lowry et al., 1951 method [58].

Quantification of Total Cytochrome P450 content

Total cytochrome P450 content of the microsomal fraction was measured using the ferrous CO verses ferrous difference Spectrum according to the method by Guengerich et al., 2009 [59]. 400µL of the sample was diluted with 600µL of solubilization buffer in two separate 1cm quartz cuvettes. The buffer consisted of 100mM potassium phosphate buffer (pH 7.4) containing 0.1mM EDTA, 20% glycerol, 0.5% sodium cholate and 0.5% Triton X100 (non-ionic detergent). About 1mg of sodium dithionite was added to both cuvettes. The cuvettes were covered with parafilm and inverted without vigorous shaking to mix the contents. Both cuvettes were placed in a UV-2400 PC Series spectrophotometer (Shimadzu, China)

and the baseline between 400 and 500nm was recorded. The sample cuvette was removed from the spectrophotometer and CO gas was slowly bubbled through the sample at the bottom of the cuvette for thirty seconds at one bubble per second in a fume hood. The sample cuvette was placed back to its original position in the spectrophotometer and the absorbance was recorded between 400 and 500 nm for several minutes. The Cytochrome P450 concentration was calculated using the following formula according to reference [59]:

$(\Delta A_{450} - \Delta A_{490}) / 0.091 = \text{nmol of P450 per mL}$, where 0.091 is the Extinction Coefficient ($\Delta \epsilon_{450-490}$). The final concentration was expressed as P450 per mg protein.

Date analysis

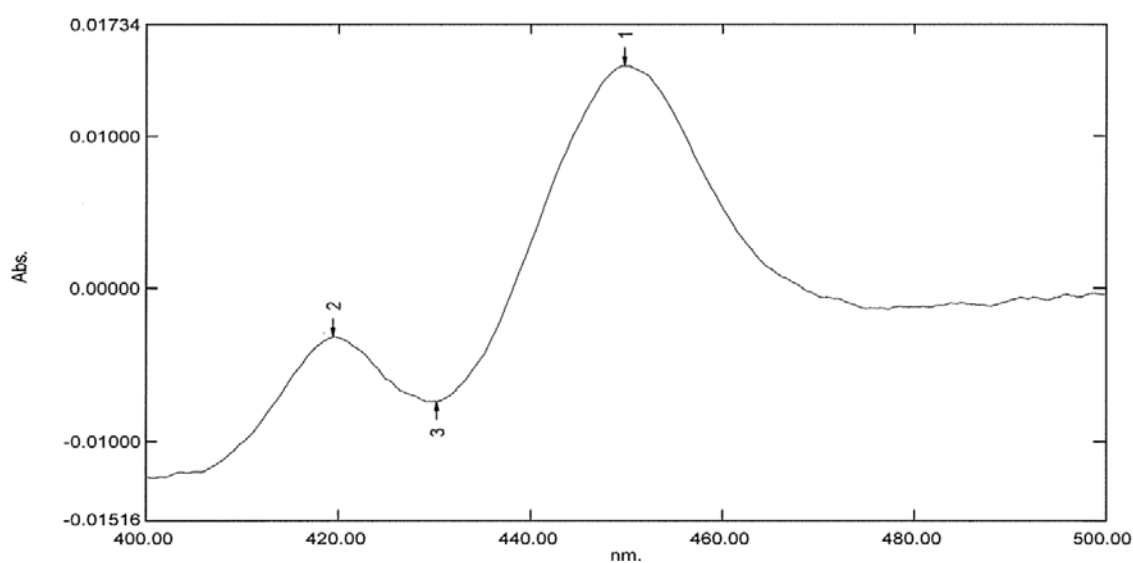
The results of the Ames test was interpreted using a non-statistical method based on the 2-fold rule by Mortelmans and Zeiger, 2000 [56]. An extract is considered mutagenic (positive mutagen) if it produces a 2-fold or more dose related increase in revertant colonies compared to that of the negative control (spontaneous mutation). The extract is considered to be non-mutagenic if there is no dose-related 2-fold increase in revertants and inconclusive where there is slight increase in revertants but cannot be considered positive or negative.

Results

Total Cytochrome P-450 Quantification test

The result of the total cytochrome P-450 measurement is shown on Figure 1 A and B. Figure A represents S9 fraction only and Figure B is S9 activated by rifampin.

A)



B

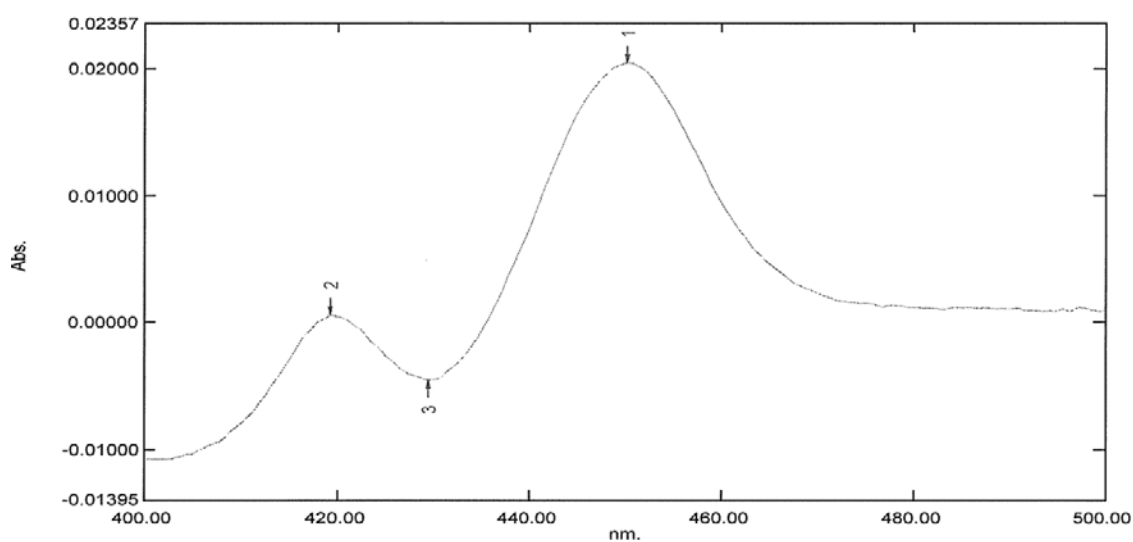
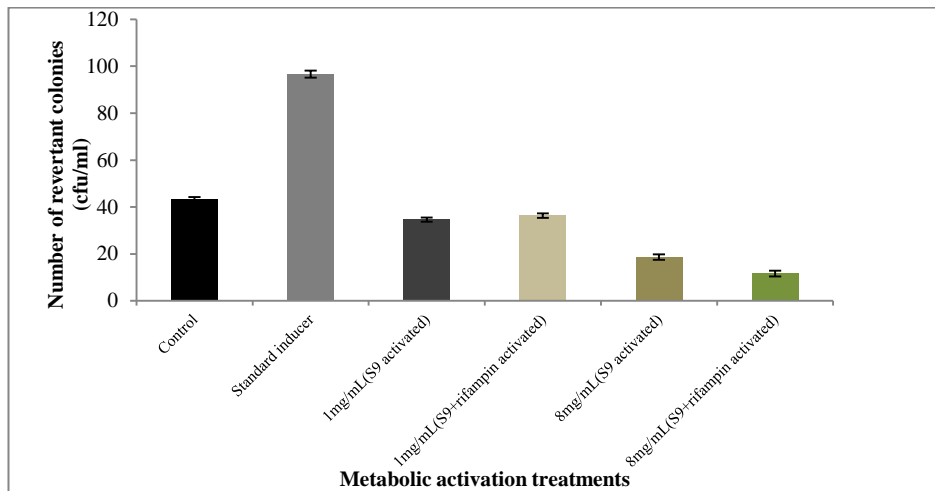


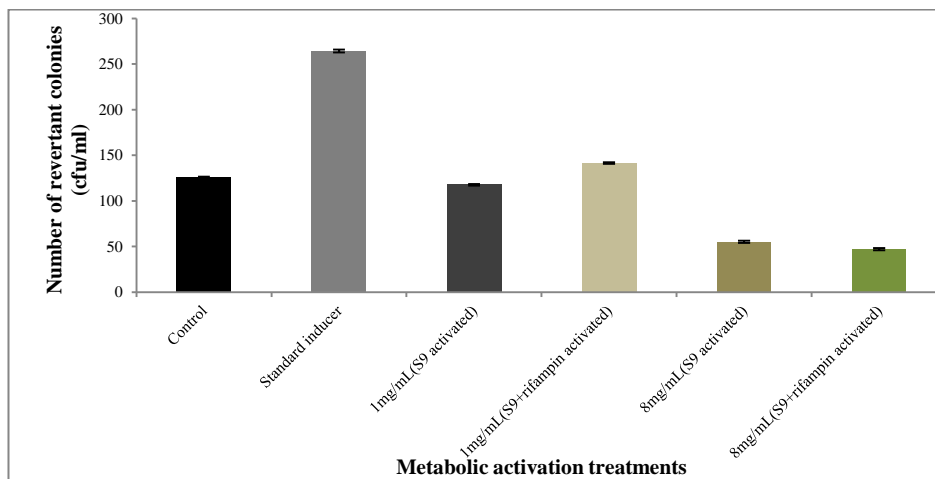
Fig 1. Absorbance spectrum of the microsomal fraction showing absorbance peak at 450nm (wavelength) with S9 only (A) and S9+Rifampin (B)

The fact that the total quantity of P450 was increased from 1.063 nmol / mg protein for non-perfused S9 microsomal fraction prepared from pig livers to 1.777 nmol / mg protein for livers perfused with rifampin indicates activation of microsomal enzymes.

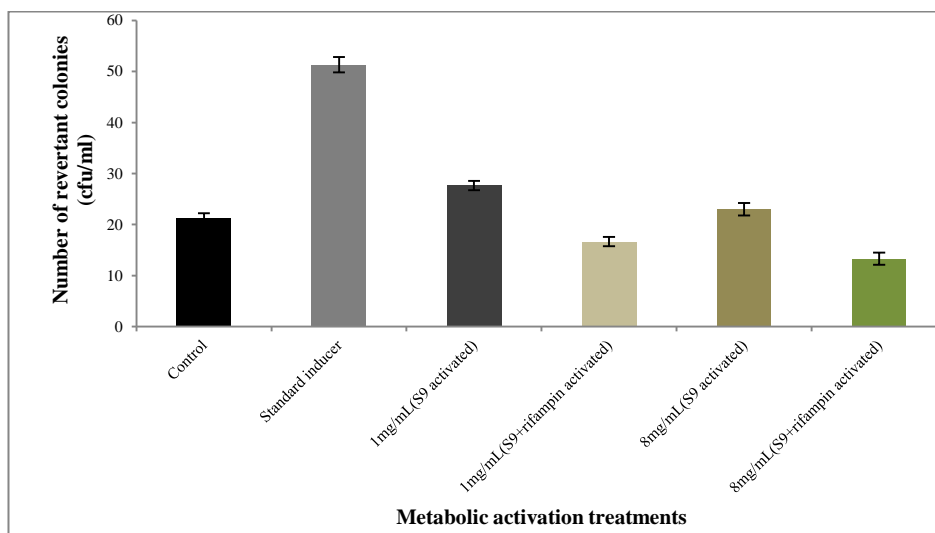
Ames test results



A

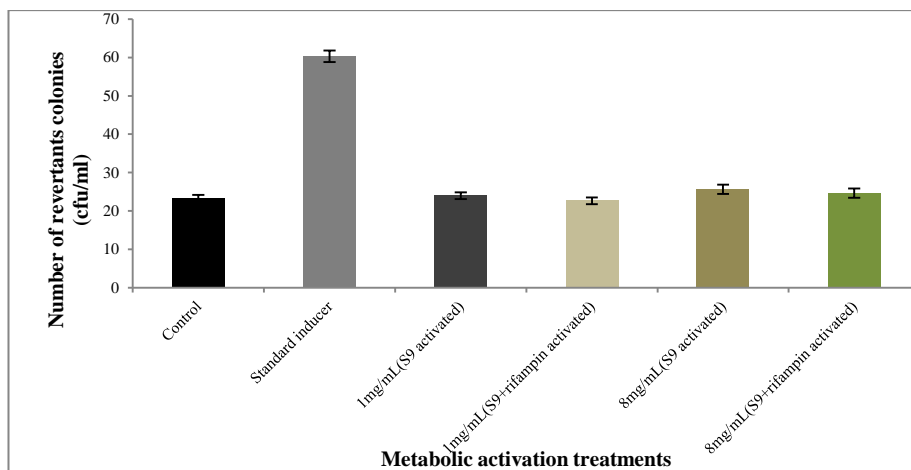


B

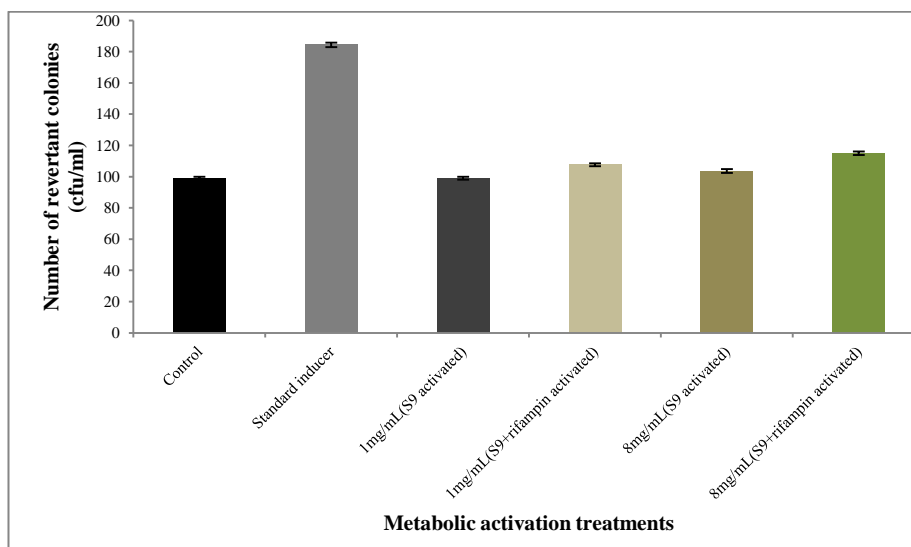


C

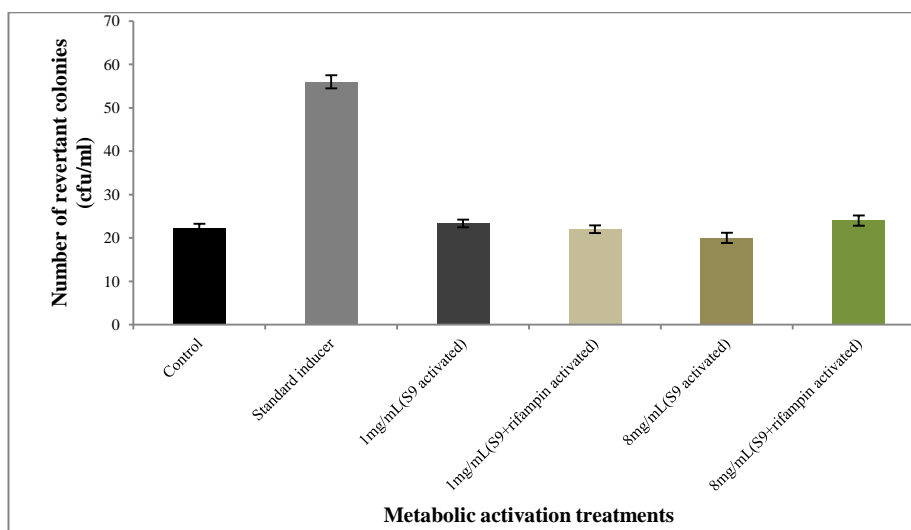
Figure 2: Extract E8 showing anti-mutagenicity outcome compared to control at 8mg/mL for S9 activated and S9+Rifampin activated P450 with *S. typhimurium* strains TA98(A), TA100 (B) and a negative mutagenicity outcome for TA1537(C). Standard inducers: 2-aminoanthracene (10µg/plate). The values are represented as mean±SEM for three replicates



A



B



C

Figure 3: Mutagenicity of extract E1 showing absence of his revertants (negative outcome) of S9 activated versus S9+Rifampin activated P450 using TA98(A), TA100 (B) and TA1537 (C). Standard inducer, 2-aminoanthracene (10 μ g/plate). The values are represented as mean \pm SEM for three replicates

Discussion

Results from the Ames test of the Ghanaian medicinal plant products did not indicate any positive mutagenic effect on the mutant strains used for test. However, extract E8 which had been prepared by the herbal manufacturer from aqueous mixture of *Clausena anisata*, *Nauclea latifolia*, *Khaya senegalensis*, *Piliostigma thonningii* and *Trichilia monadelpha* was found to produce a 2-fold decrease in revertant colonies compared to the negative controls in both the metabolic activation systems of TA98 and TA100. Clearly, the antimutagenicity activity must be confined to one or more medicinal plants used for the preparation of E8. This was, a clear indication of the potential antimutagenicity of extract E8. Methanol lominoid fraction isolated from *Khaya senegalensis* had been previously reported to exhibit anti-cancer activity for human colon cancer cell lines, human breast cancer cell lines and cervical cancer cell lines at 2-200 parts per million (ppm) [60]. Significant free-radical scavenging activity of aqueous and ethanolic extracts of this medicinal plant has also been reported, using a 1,1, diphenyl-2picryl-hydrazyl (DPPH) test at 1.28 to 21µg/mL [61]. A similar antioxidant and free-radical scavenging activity of *K. senegalensis* methanol extract at 500mg/mL has also been reported Atawodi et al., 2009 [62]. On the other hand, an alkaloid rich chloroform fraction isolated from *Nauclea latifolia* was reported to have produced some mutagenicity in the Ames spot test with TA 97 and TA 100 at 10µg. Furthermore, the fraction exhibited an anti-proliferative activity on human monocytes at the same concentration [13]. Hydro-methanolic saponin fractions isolated from the plant has also been association with DNA-damage and chromosome mutations in Chinese Hamster ovary cells at 2-10µg [63]. *Clausena anisata* fraction, chalepin was reported to be non-mutagenic in an Ames spot test at 500µg [64]. However, the ethanol extract of *Piliostigma thonningii* has been reported to have antioxidant and free radical scavenging activity *in vivo* against carbon tetrachloride-induced hepatic and oxidative damage in Albina rats at 10, 100 and 200mg/kg body weight [65]. Methanol extract of the plant was also non-toxic at 10-5000µg/mL in a brine shrimp lethality assay [66]. Extracts of *Trichilia monadelpha*, ethyl acetate, petroleum ether and ethanol also exhibited free radical scavenging activity at 0.1-3mg/mL in a DPPH and reducing power assay [67]. Although *Nauclea latifolia* is reported to have some mutagenic activity, its toxic effect in E8 appears to have been suppressed potentially by unknown interactions with the bioactives

produced by the other four plants in the extract. The antimutagenic results obtained from the test could be attributed to the reported antioxidant activities of the four plant components in the mixture. Other studies have shown a correlation between antioxidant and free radical scavenging activity of plants and their anti-mutagenicity. ([68], [69]).

It is now important to determine the medicinal plant constituent (s) of E8 aqueous extract responsible for the antimutagenic activity. It is also important that after identification of the plant constituent responsible for mutagenicity, further tests on cytotoxicity are conducted using a selection of human cell lines to determine the anti-cancer properties of E8.

Conclusion

The aim of this study was to screen commonly marketed Ghanaian medicinal plant products for mutagenicity using the bacterial reverse mutation assay (Ames test). None of the products tested indicate any positive mutagenic effect, whilst extract E8 was found to have exhibited an antimutagenic effect. Further studies are warranted to identify the plant constituent (s) responsible for the antimutagenicity of E8 and cytotoxicity test conducted to determine the anti-cancer properties of the extract E8.

Conflict of Interest

The authors declare no conflict of interest

Acknowledgement

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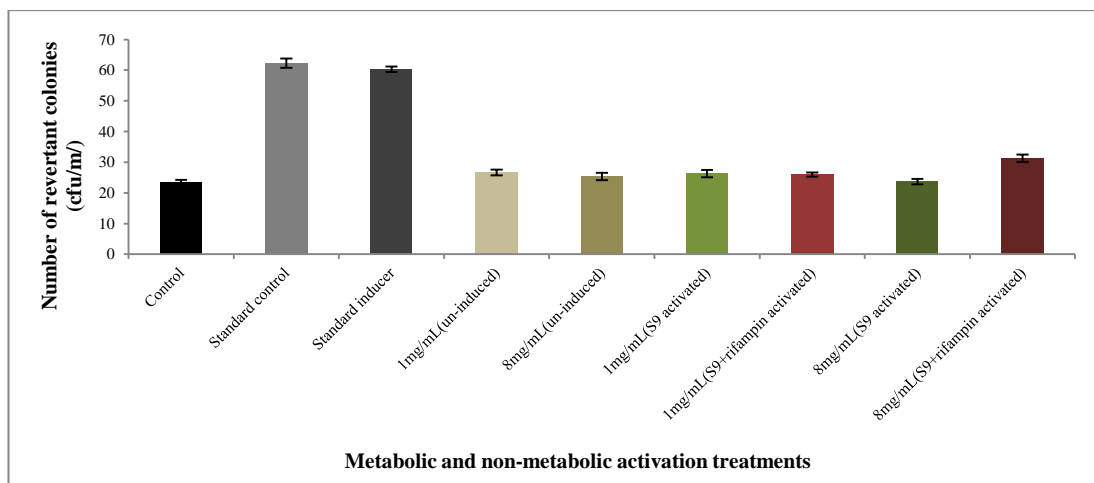
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Supplementary Information for Commonly Marketed Herbal Extract Formulations Demonstrating Negative Outcomes in the Ames test

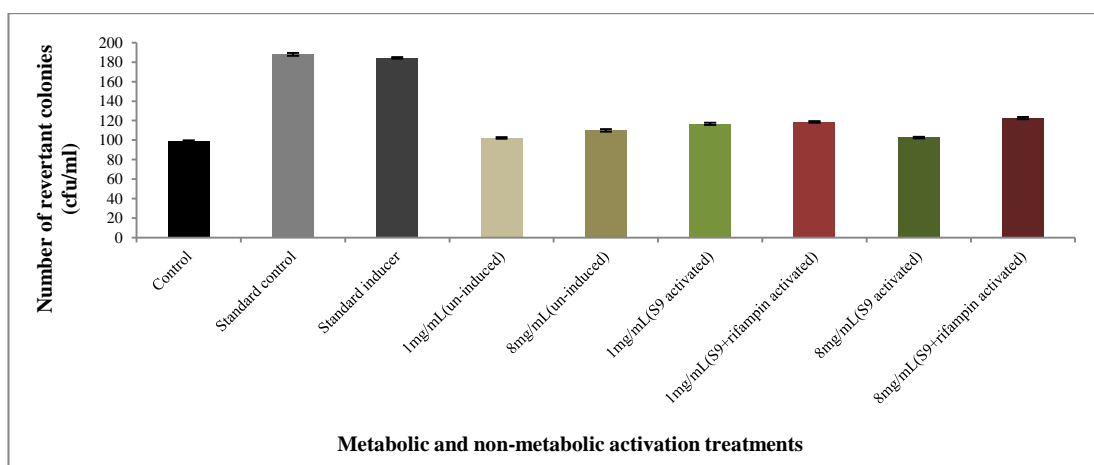
The Ames test results are interpreted using the 2-fold rule by Mortelmans and Zeiger (2000) as stated in chapter three. Accordingly, an extract is considered mutagenic (positive mutagen) if it produces a 2-fold or more increase in revertant colonies compared to that of the negative control (spontaneous mutation), and negative for mutagenicity if there is no 2-fold or more increase in revertant colonies over negative control. Ames test results for Extracts E2, E4, E5, E6, E7, E9 and E10 which were found to be negative for mutagenicity. The results are presented on Figures 4 A-C to 10 A-C.

4.1 Extract E2

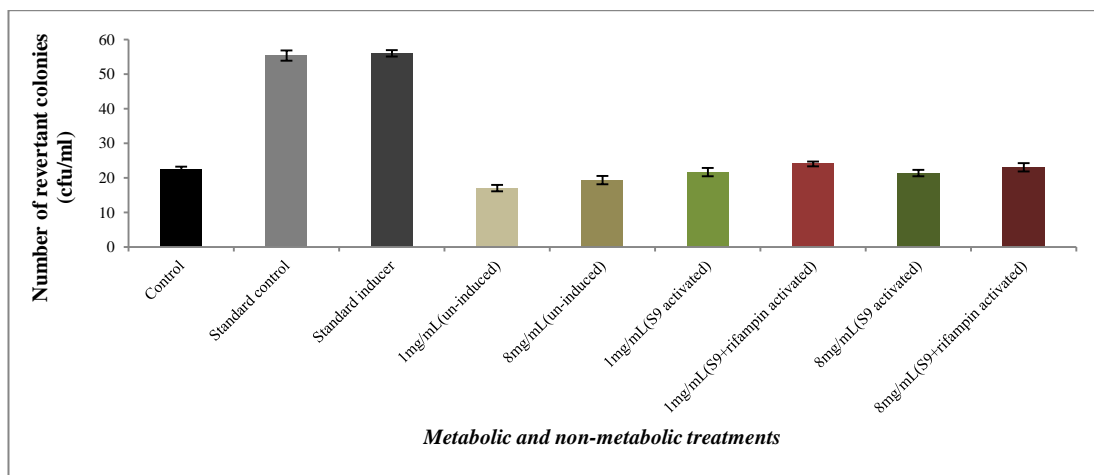
Results of Extract E2 for both un-induced (without metabolic activation) and induced (S9 activated and S9+Rifampin activated) are presented in Figure 4 for all three *S. typhimurium* strains tested (TA98, TA100 and TA1537). Compared to their controls, TA98 (A), TA100 (B) and TA1537(C) did not show any 2-fold increases in revertant colonies at both concentrations of 1mg/mL and 8mg/mL (Fig A-C), and hence not mutagenic. The CFUs were not 2 fold lower than the controls and hence were not antimutagenic.



A



B

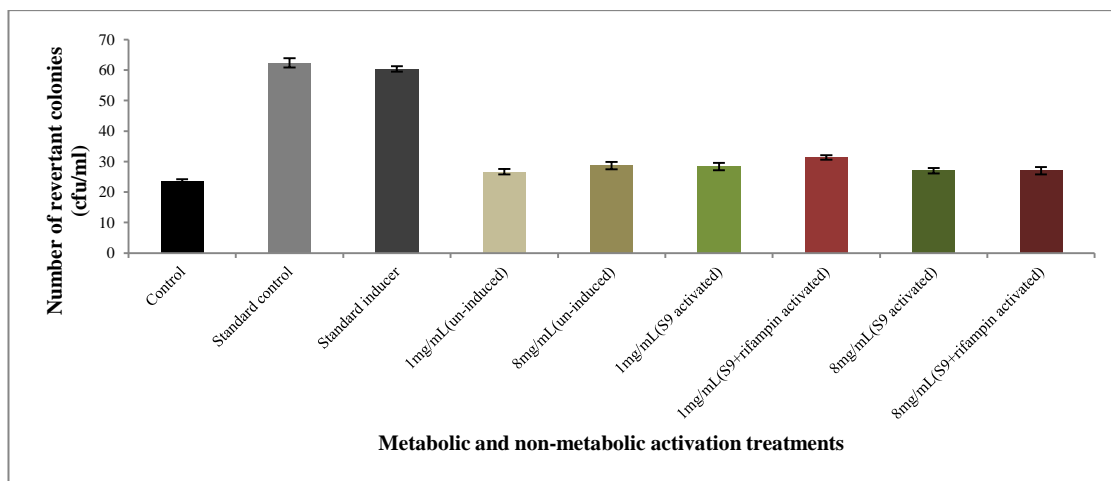


C

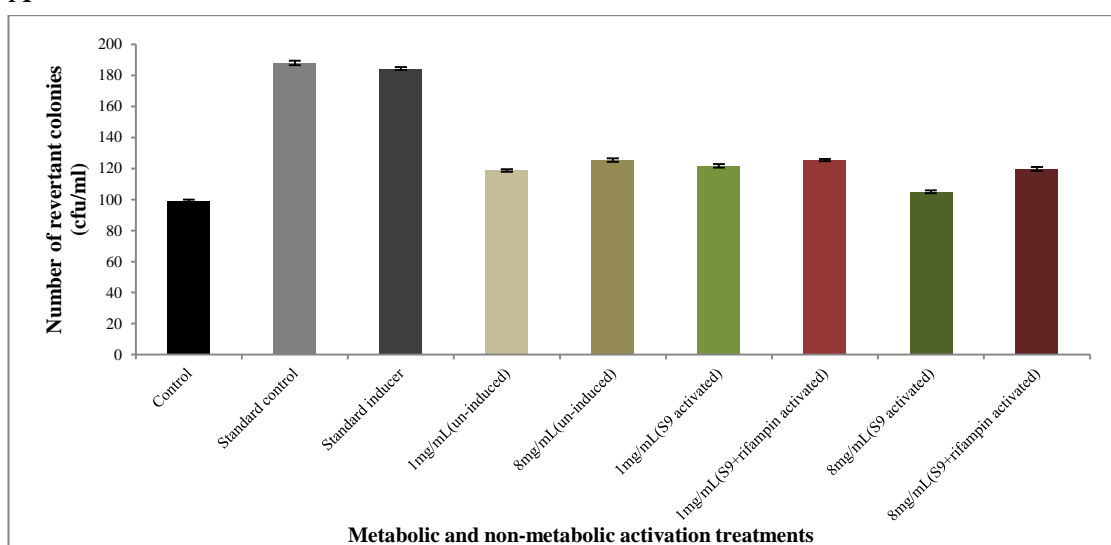
FIG 4: Extract E2 showing negative mutagenicity outcomes compared to control for TA98(A), TA100(B) and TA1537(C). Standard controls, without metabolic activation (un-induced) = Acridine orange, 10 μ g/plate (A), Sodium azide, 10 μ g/plate (B), Neutral red, 10 μ g/plate (C). Standard inducer, with metabolic activation = 2-aminoanthracene, 10 μ g/plate. The values are represented as mean \pm SEM for three replicates

4.2 Extract E4

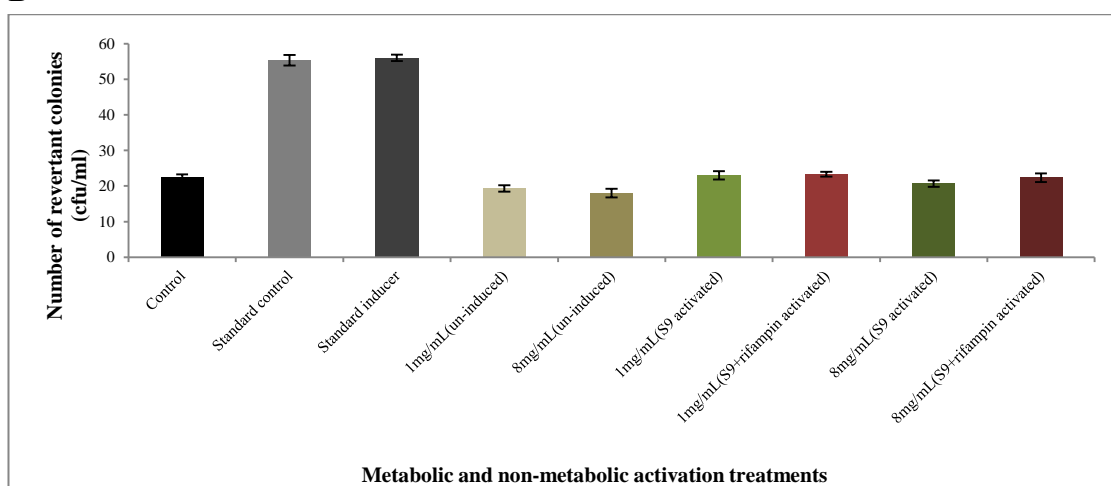
Extract E4 results for both un-induced (without metabolic activation) and induced (S9 activated and S9+Rifampin activated) are presented in Figure 5 for all three *S. typhimurium* strains tested (TA98, TA100 and TA1537). TA98 (A) and TA1537(C) did not show any 2-fold increases in revertant colonies as compared to their control at both concentrations of 1mg and 8mg in all test systems. An increase in reversion was observed for TA100 (B) at both concentrations for all test systems; however, these increases were not up to 2-fold that of the control, and hence not mutagenic. The CFUs were not 2 fold lower than the controls and hence were not antimutagenic.



A



B

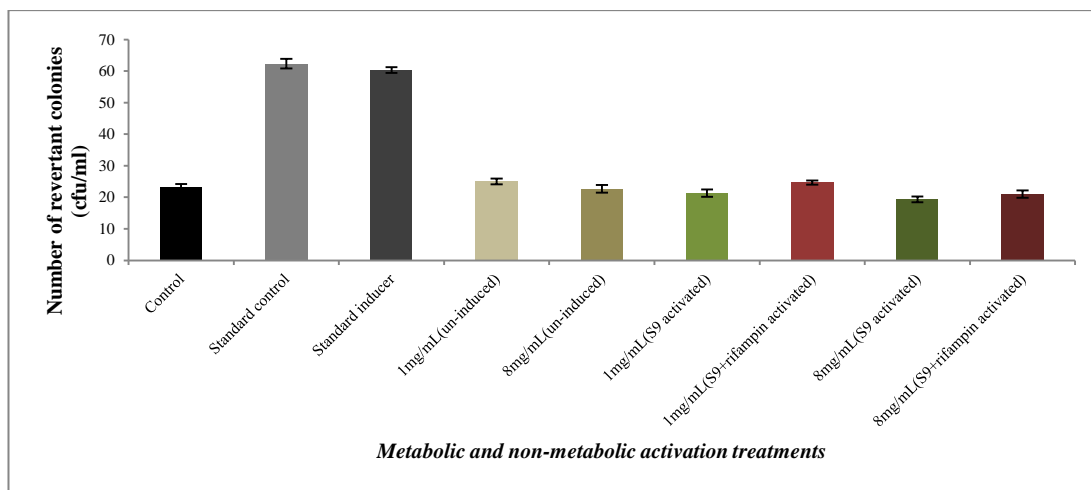


C

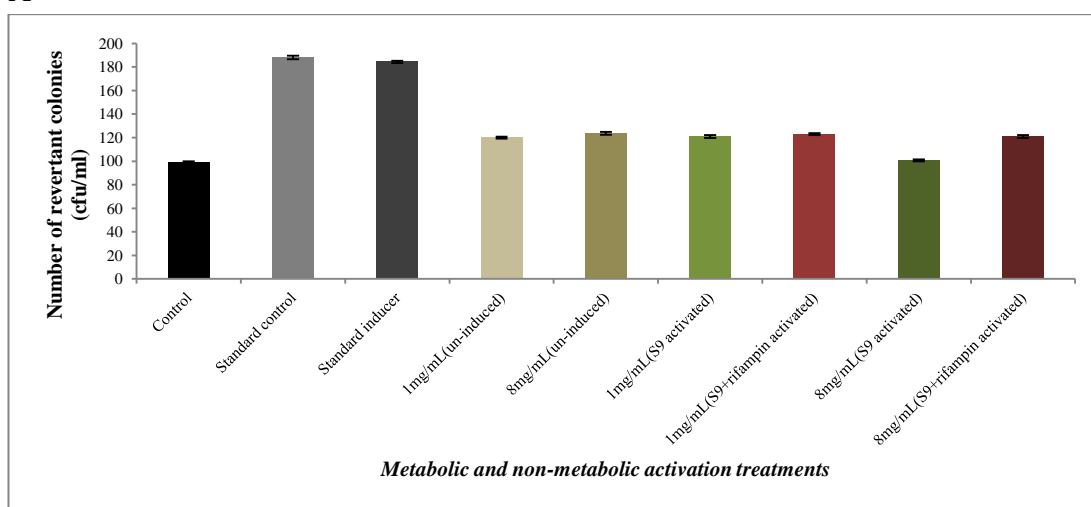
FIG 5: Extract E4 showing negative mutagenicity outcomes compared to control for TA98(A), TA100(B) and TA1537(C). Standard controls, non-metabolic activation (un-induced) = Acridine orange, 10 μ g/plate (A), Sodium azide, 10 μ g/plate (B), Neutral red, 10 μ g/plate (C). Standard inducer, with metabolic activation = 2-aminoanthracene, 10 μ g/plate. The values are represented as mean \pm SEM for three replicates.

4.3 Extract E5

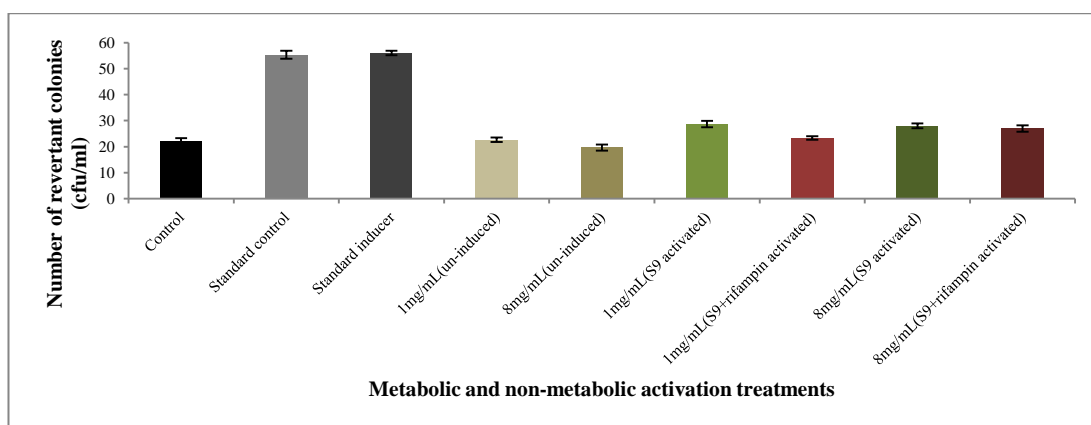
Extract E5 results for both un-induced (without metabolic activation) and induced (S9 activated and S9+Rifampin activated) are presented in Figure 6 for all three *S. typhimurium* strains tested (TA98, TA100 and TA1537). TA98 (A) and TA1537(C) did not show any 2-fold increases in revertant colonies as compared to their control at both concentrations of 1mg and 8mg in all test systems. There were slight increases in reversion for TA100 (B) at both concentrations for all test systems, but these increases were not up to 2-fold that of the control, and hence not mutagenic. The CFUs were not 2 fold lower than the controls and hence were not antimutagenic.



A



B

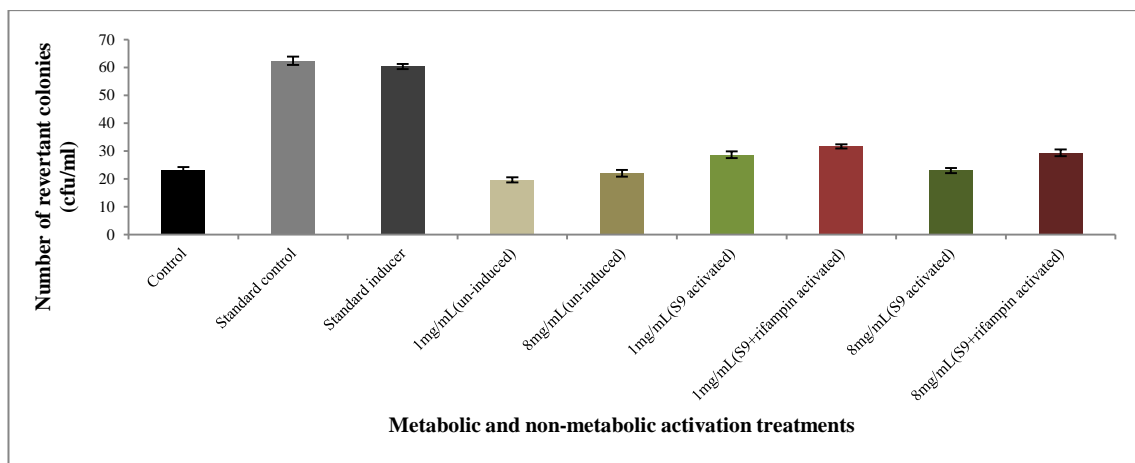


C

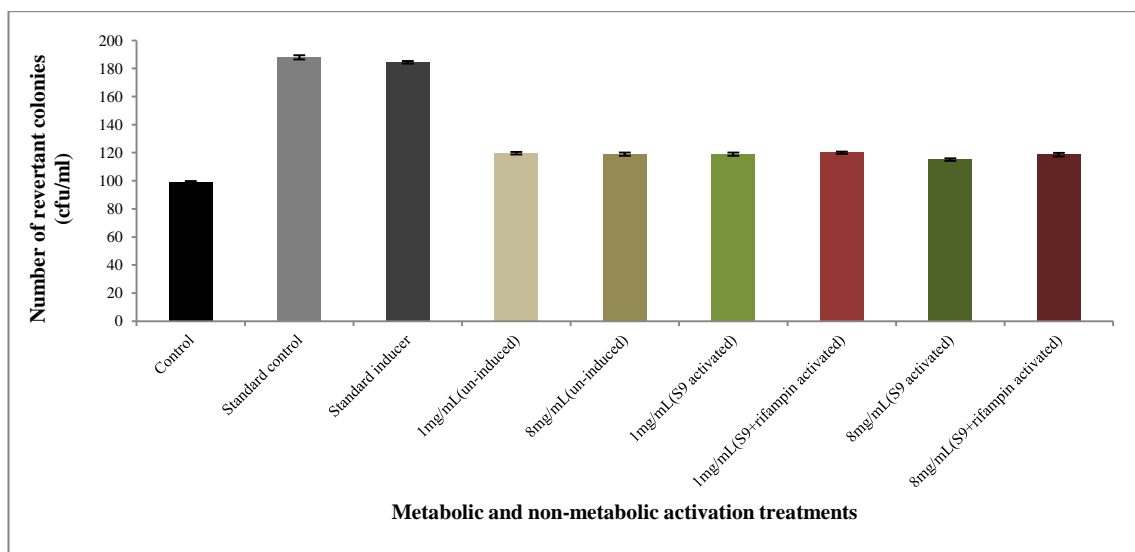
FIG 6: Extract E5 showing negative mutagenicity outcomes compared to control for TA98(A), TA100(B) and TA1537(C). Standard controls, non-metabolic activation (un-induced) = Acridine orange, 10 μ g/plate (A), Sodium azide, 10 μ g/plate (B), Neutral red, 10 μ g/plate (C). Standard inducer, metabolic activation = 2-aminoanthracene, 10 μ g/plate. The values are represented as mean \pm SEM for three replicates

4.4 Extract E6

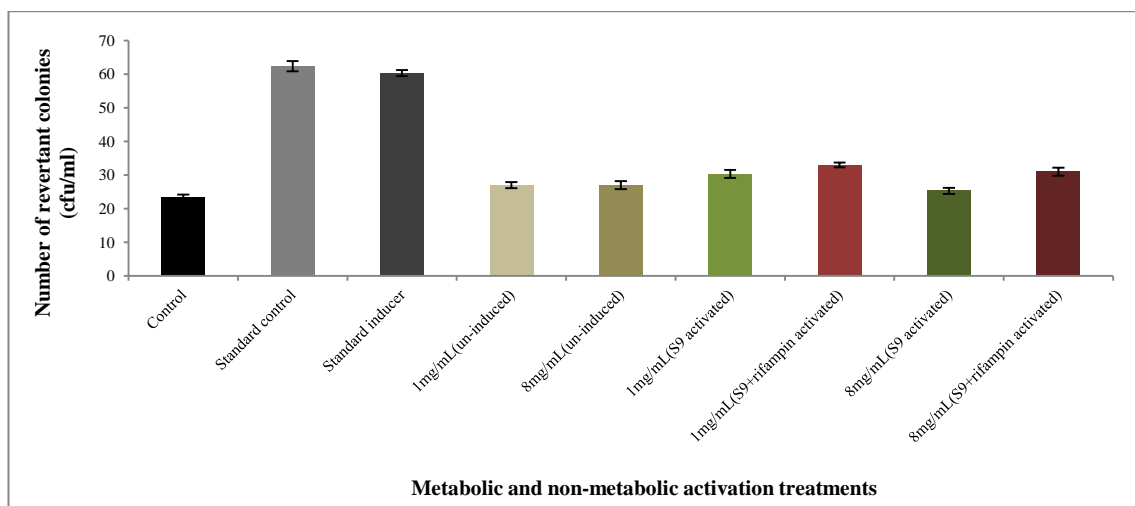
The results for extract E6 results for are presented in Figure 7 for all three *S. typhimurium* strains tested (TA98, TA100 and TA1537) for un-induced (without metabolic activation) and induced (S9 activated and S9+Rifampin activated). Although some slight increases in reversion was observed for TA98 induced (A), TA100 and TA1537, these increases were not up to 2-fold that of the control spontaneous revertant colonies, and hence not mutagenic. The CFUs were not 2 fold lower than the controls and hence were not antimutagenic.



A



B

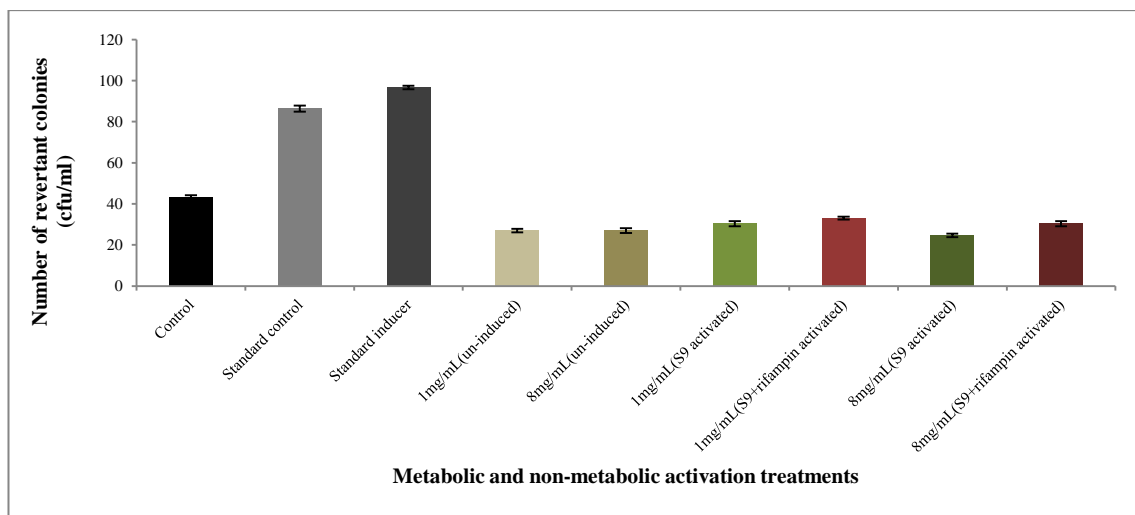


C

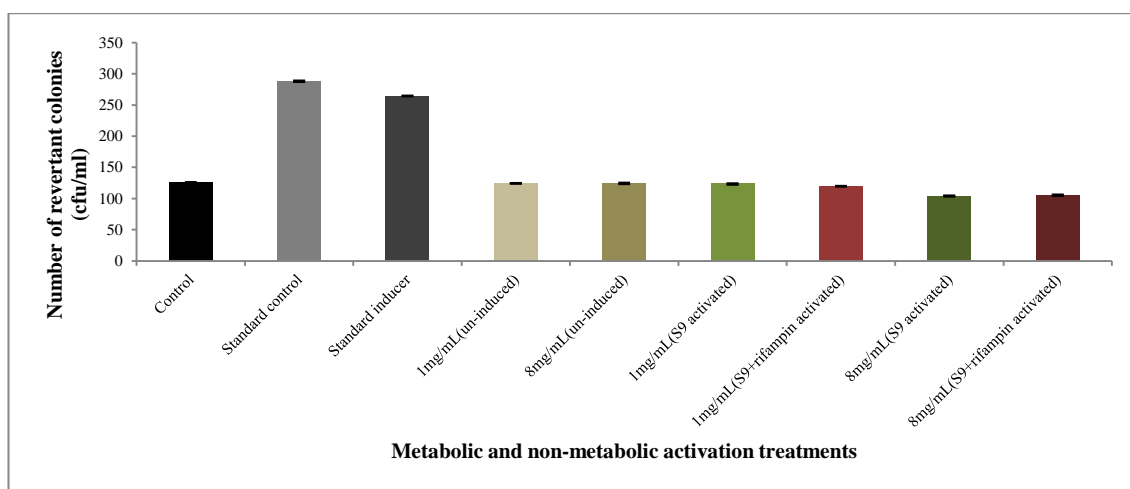
FIG 7: Extract E6 showing negative mutagenicity outcomes compared to control for TA98(A), TA100(B) and TA1537(C). Standard controls, non-metabolic activation (un-induced) = Acridine orange, 10 μ g/plate (A), Sodium azide, 10 μ g/plate (B), Neutral red, 10 μ g/plate (C). Standard inducer, metabolic activation = 2-aminoanthracene, 10 μ g/plate. The values are represented as mean \pm SEM for three replicates.

4.5 Extract E7

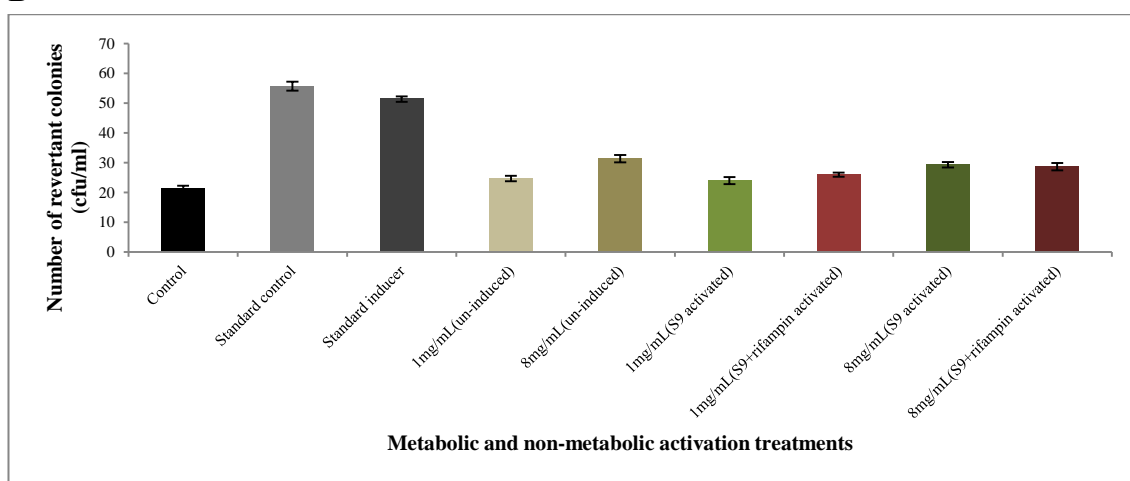
Results for Extract E7 are presented in Figure 8 for both un-induced (without metabolic activation) and induced (S9 activated and S9+Rifampin activated). Compared to their controls, TA98 (A) and TA100 (B) did not show any 2-fold increases in revertant colonies at both concentrations of 1mg and 8mg in all test systems. Slight increases were observed at 8mg/mL for all test systems for TA1537 (C), however, these increases were not up to 2-fold as compared to the control revertant colonies, and hence not mutagenic. The CFUs were not 2 fold lower than the controls and hence were not antimutagenic.



A



B

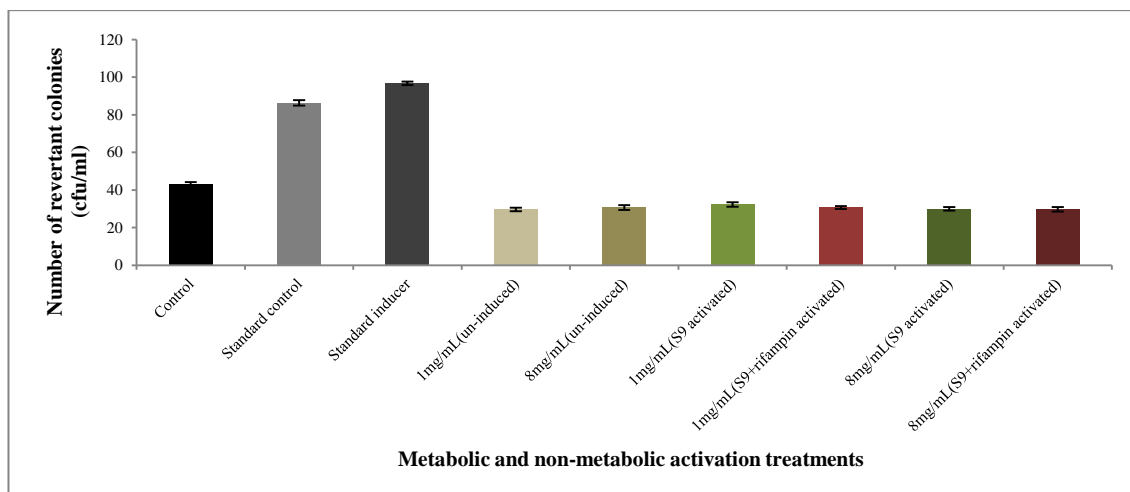


C

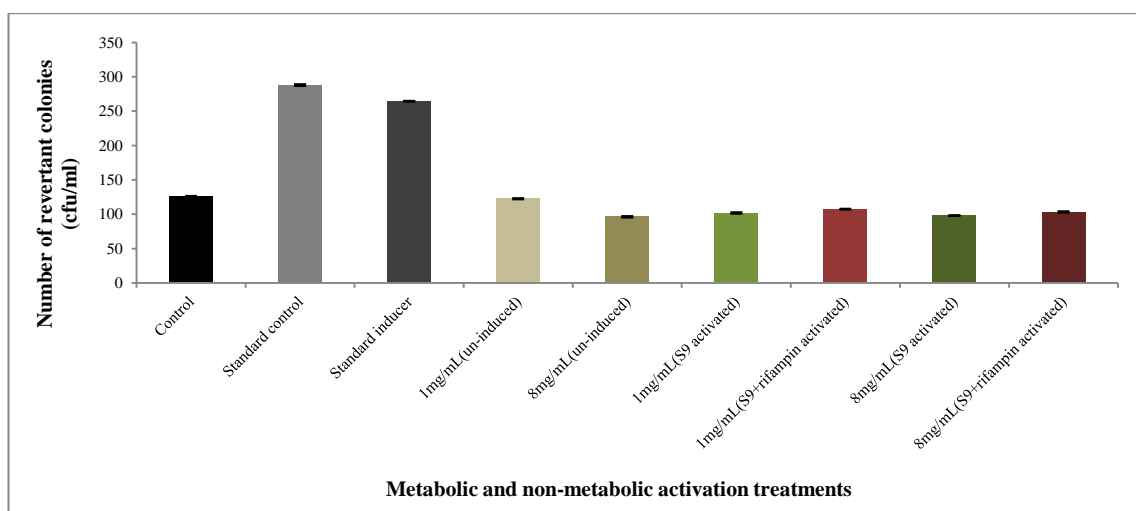
FIG 8: Extract E7 showing negative mutagenicity outcomes compared to control for TA98(A), TA100(B) and TA1537(C). Standard controls, non-metabolic activation (un-induced) = Acridine orange, 10 μ g/plate (A), Sodium azide, 10 μ g/plate (B), Neutral red, 10 μ g/plate (C). Standard inducer, metabolic activation = 2-aminoanthracene, 10 μ g/plate. The values are represented as mean \pm SEM for three replicates.

4.6 Extract E9

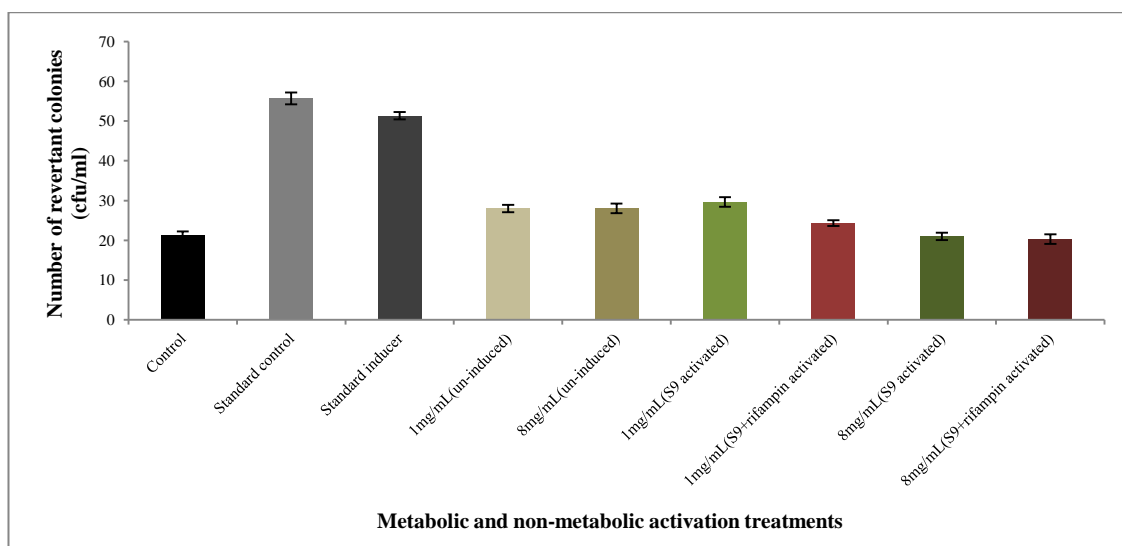
The Ames test results for Extracts E9 for both un-induced (without metabolic activation) and induced (S9 activated and S9+Rifampin activated) are shown on Figure 9. TA98 (A) and TA100 (B) did not show any 2-fold increases in revertant colonies at both concentrations whilst there were slight increases of revertants at 1mg and 8mg/mL for TA1537 (C) un-induced and at 1mg S9 activated. These increases were not up to 2-fold that of the control spontaneous revertants, and hence not mutagenic. The CFUs were not 2 fold lower than the controls and hence were not antimutagenic.



A



B

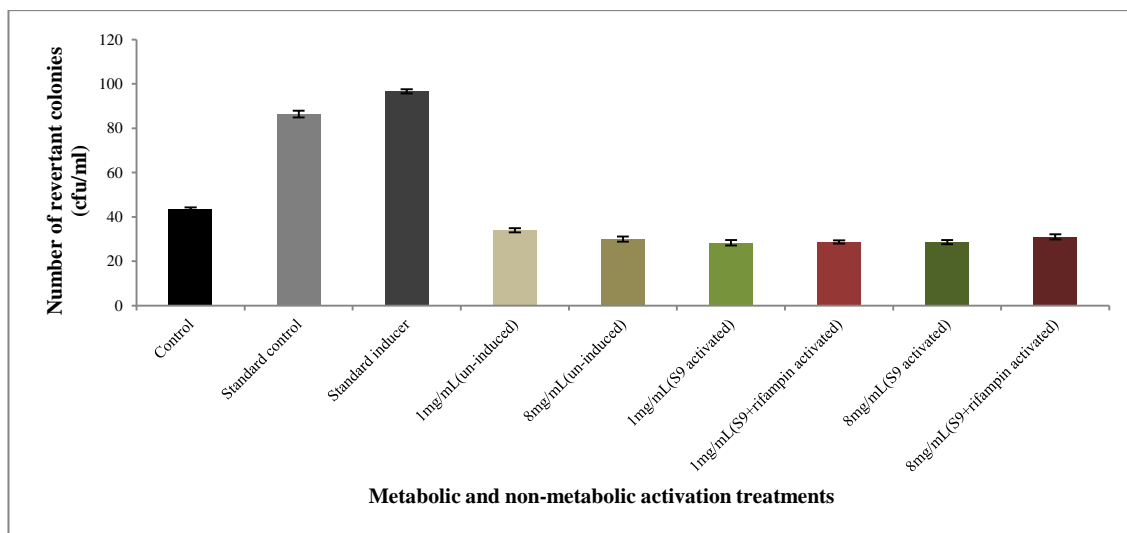


C

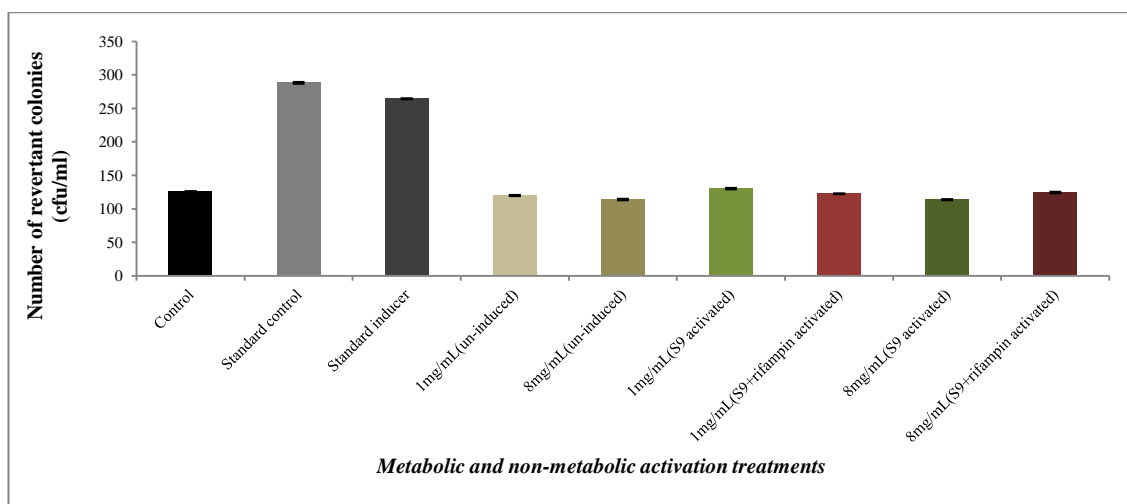
FIG 9: Extract E9 showing negative mutagenicity outcomes compared to controls for TA98(A), TA100(B) and TA1537(C). Standard controls, non-metabolic activation (un-induced) = Acridine orange, 10 μ g/plate (A), Sodium azide, 10 μ g/plate (B), Neutral red, 10 μ g/plate (C). Standard inducer, metabolic activation = 2-aminoanthracene, 10 μ g/plate. The values are represented as mean \pm SEM for three replicates.

4.7 Extract E10

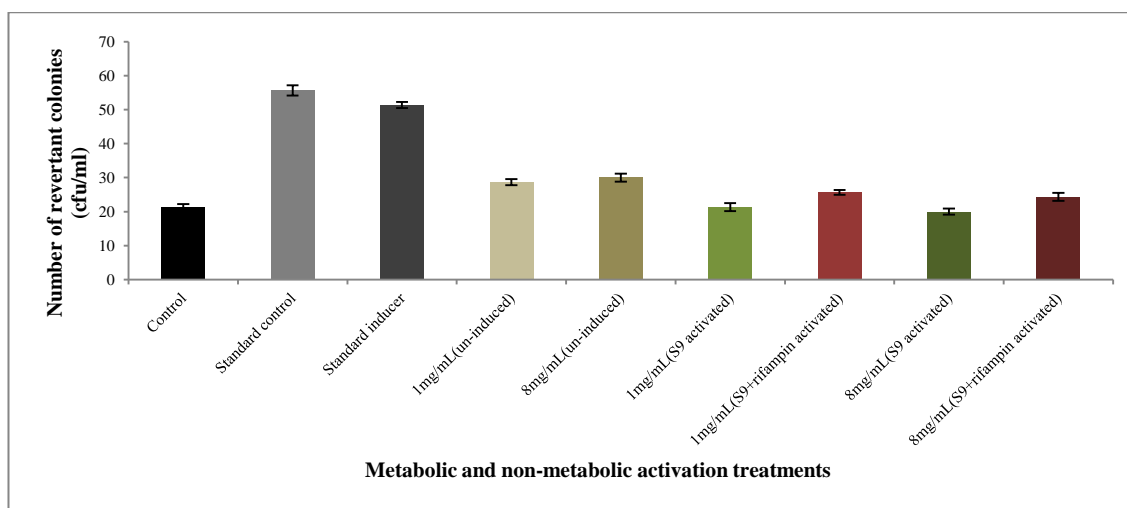
The Ames test results for Extracts E10 for both un-induced (without metabolic activation) and induced (S9 activated and S9+Rifampin activated) are shown on Figure 10. TA98 (A) and TA100 (B) did not show any 2-fold increases in revertant colonies at both concentrations. However, there were slight increases observed at 1mg and 8mg/mL for TA1537 un-induced and at 8mg/mL S9+Rifampin activated which were not up to 2-fold that of the control spontaneous revertants.



A



B



C

FIG 10: Extract E10 showing negative mutagenicity outcomes compared to control for TA98(A), TA100(B) and TA1537(C). Standard controls, non-metabolic activation (un-induced) = Acridine orange, 10 μ g/plate (A), Sodium azide, 10 μ g/plate (B), Neutral red, 10 μ g/plate (C). Standard inducer, metabolic activation = 2-aminoanthracene, 10 μ g/plate. The values are represented as mean \pm SEM for three replicates.

**Antimicrobial Activity of Ghanaian Medicinal Plant
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Antimicrobial Activity of Ghanaian Medicinal Plant Products

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Abstract

Nine Ghanaian medicinal plant products commonly sold on the Ghanaian marketplace were tested for their antimicrobial activity on selected clinical bacterial isolates at a concentration of 2mg/mL, using the agar well diffusion method. From the results, one out of the nine products screened (E8) exhibited antimicrobial activity against *Moraxella catarrhalis*, giving a mean inhibition zone diameter of 20.7 ± 1.2 mm. The product is prepared by the herbal manufacturer from combination of five different medicinal plants (*Khaya senegalensis*, *Nauclea latifolia*, *Pilostigma thonningii*, *Trichilia monadepha* and *Clausena anisata*). All other products showed no antimicrobial activity (resistant) against any of the pathogens tested. Ciprofloxacin (2.5µg disc) was the most sensitive antibiotic control giving inhibition zone diameter of 40 ± 1 mm (*E.coli*), followed by 35.7 ± 1.2 mm (*S. typhimurium*), 35 ± 1 mm (*B. paraptussis*) and 32 ± 2 mm (*M. catarrhalis*). The minimum inhibitory concentration (MIC) of E8 was 125µg. The minimum bactericidal concentration of E8 was 500µg whereas that of the antibiotic controls was 125µg for both Cephalexin and Trimethoprim.

Keywords: Medicinal plants, antimicrobial activity, *Moraxella catarrhalis*, Ghanaian medicinal plants, Agar well diffusion test

Introduction

Herbal medicine is widely used, especially in developing countries because it is readily available and affordable. In Ghana, most people depend on herbal medicine to meet their basic healthcare needs. It has been found that most plants used in the preparation of herbal medicine contain bio-active compounds which are usually extracted for human use. Plants can be used in their raw form or as extract (crude extract) for medication, a common practice in most developing countries ([1], [2]). Herbal medicine plays a significant role in Africa, and has also maintained its popularity worldwide. About 80% of African and Asian population including Ghana use herbal remedies as alternative to orthodox medicine for their primary health care. Whilst in the developed countries, 70% to 80% of the population has used complementary or alternative medicine. Herbal medicine is usually the first line of treatment for about 60% of children with malaria in countries like Ghana, Nigeria, Zambia and Mali [3]. This is because herbal medicine is easily accessible, affordable and is ingrained in their everyday life and well-being. Almost all parts of plants are used for medicinal purposes, especially the roots, whole plants, barks, flowers, leaves and stem. Medicinal plants have many uses, such as antimicrobials ([4], [5]), antidiabetic ([6], [7], [8]), anti-atherogenic and anti-ischemic [9], as antioxidants ([10], [11]), anticancer [12], and as anti-malaria ([13], [14], [15], [16]). In Ghana, it is estimated that 951 tons of crude plant medicine (excluding pre-packed herbal medicine) are sold annually on the Ghanaian domestic market, amounting to a total of US\$7.8 million market value and an export value of US\$15 million [17]. Considering the current trend of antibiotic resistance and the availability of plants with bio-active compounds, plant based medicine could serve as alternative to conventional medicine. The aim of the study is to screen some selected medicinal plant products (pre-packaged) commonly sold on the Ghanaian marketplace for their antimicrobial activities.

Materials And Methods

Medicinal Plant Products

The medicinal products used were pre-prepared as herbal medicines which were purchased from the Ghanaian market in Accra, Ghana. The products were all

aqueous extracts indicated for conditions such as malaria, typhoid fever, diabetes, cough, piles, candidiasis, asthma and anaemia.

Table 1 Medicinal products and their plant constituents

Extract	Plant Constituents
E1	<i>Nauclea latifolia, Phyllanthus fraternus, Cryptolepis sanguinolenta</i>
E2	<i>Ocimum gratissimum, N. latifolia, C. sanguinolenta</i>
E4	<i>Cnestis ferruginea, Paullinia pinnata</i>
E5	<i>Momordica charantia Vernonia amagdalina, Strophanthus hispidus, Lippia multiflora</i>
E6	<i>Khaya senegalensis, Kigelia africana, Mitragyna stipulosa</i>
E7	<i>Monodora myristica, Chlorophora excelsa, Alchornea cordifolia, K. senegalensis</i>
E8	<i>Clausena anisata, Pilostigma thonningii, Trichilia monadelpha, K. senegalensis, N. latifolia</i>
E9	<i>Anthoscleista nobilis, Mussaenda erythrophylla, Vernonia amygdalina</i>
E10	<i>Desmodium adscendens</i>

E = EXTRACT

Micro-organisms

The micro-organisms used for the test were clinical isolates obtained from the Microbiology Service Section, School of Biomedical Sciences, Curtin University. They consisted of *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Moraxella catarrhalis*, *Bordetella parapertussis*, *Acinetobacter baumannii*, *Salmonella typhimurium* and *Candida albicans*.

Agar Diffusion Test

The antimicrobial activity was performed by the agar-well diffusion method [18]. The bacteria strains were inoculated into 3.5mL of sterile saline solution from overnight-growth cultures on Mueller Hinton and blood agar. The turbidity of the bacterial suspensions was adjusted to 0.5 McFarland standards (1.5×10^8 cfu/mL). Lawn inoculum of the various bacteria strains were made on their respective agar

plates using sterile cotton swabs. A sterile cork borer of 6mm diameter was used to create wells on the agar plates and 100µL of each aqueous extracts were added to their respective labelled wells. The plates were refrigerated at 4°C for 2 hours to allow diffusion of extracts into the medium before incubation at 37°C for 24 hours. All tests were done in triplicates. Cephalixin and trimethoprim were used as control antibiotics for validation of the assay. Antibigrams of the pathogens was also determined using the calibrated dichotomous sensitivity test (CDS) as described by Bell, 1975 [19]. Antibiotic disks for this test were purchased from Sigma-Aldrich (Australia).

Determination of MIC and MBC of the Commercial Ghanaian Medicinal Plant Extracts

The minimum inhibitory concentration (MIC) was determined by the 96-well microdilution method ([20], [21]). Using 100µL/well, a two-fold dilution of the extract was made from the stock (20mg/mL) to a concentration of 2mg (2000µg, first well) to 0.002mg (1.953µg, eleventh well). Well number twelve was the solvent control, water being the control for aqueous extracts and DMSO as control for antibiotics without any extract. Trimethoprim and Cephalixin were used as the antibiotic controls at concentrations ranging from 500 to 0.488µg. The plates were sealed and incubated at 37°C for 24 hr. All tests were done in triplicate. After overnight incubation, 40µL of 0.2mg/mL p-iodonitrotetrazoliumviolet (INT) was added to all the wells and the plates examined within 30 minutes for bacterial growth. Bacteria growth was indicated by the red colour of INT reducing to formazan. The lowest concentration at which decrease in the red colour was apparent compared to the next dilution was taken as the MIC value. To determine the minimum inhibitory concentration (MBC), all wells with no visible growth were inoculated on agar plates and incubated overnight at 37°C. Plates were observed for growth after incubation, the lowest concentration at which there was no growth on the agar plate was considered the MBC.

Results

Results of the antimicrobial test of the nine aqueous extracts tested against human pathogenic bacteria are presented on Table 2. Product/extract E8 gave a significant activity against *Moraxella catarrhalis* with zone of inhibition of 20±1.2. The

antibiotic susceptibility pattern of the microorganisms is shown on Table 3. The MIC and MBC of E8 versus the control antibiotics cephalexin and trimethoprim are shown on Table 4.

Table 2

ORGANISM	E1	E2	E4	E5	E6	E7	E8	E9	E10
<i>Escherichia coli</i>	R	R	R	R	R	R	R	R	R
<i>Candida albicans</i>	R	R	R	R	R	R	R	R	R
<i>Moraxella catarrhalis</i>	R	R	R	R	R	R	S	R	R
<i>Pseudomonas aeruginosa</i>	R	R	R	R	R	R	R	R	R
<i>Acinetobacter baumannii</i>	R	R	R	R	R	R	R	R	R
<i>Streptococcus agalactiae</i>	R	R	R	R	R	R	R	R	R
<i>Salmonella typhimurium</i>	R	R	R	R	R	R	R	R	R
<i>Klebsiella pneumonia</i>	R	R	R	R	R	R	R	R	R
<i>Bordetella parapertusis</i>	R	R	R	R	R	R	R	R	R
<i>Streptococcus pyogenes</i>	R	R	R	R	R	R	R	R	R

Sensitivity test results showing resistant/sensitivity outcome of products against selected microorganisms Result = mean \pm standard deviation. R = resistant, S = sensitive, E = Extract,

Table 3

ORGANISM	E	AMP	CTX	F	CN	SXT	CIP	RD	FOX
<i>Escherichia coli</i>	NT	R	S	S	S	S	S	NT	S
<i>Candida albicans</i>	NT	NT	R	R	R	R	R	R	R
<i>Moraxella catarrhalis</i>	S	R	R	S	S	S	S	S	S
<i>Pseudomonas aeruginosa</i>	NT	R	R	R	S	R	S	NT	R
<i>Acinetobacter baumannii</i>	S	R	R	R	S	R	S	R	R
<i>Streptococcus agalactiae</i>	S	S	S	S	S	S	S	S	S
<i>Salmonella typhimurium</i>	NT	S	S	S	S	S	S	NT	S
<i>Klebsiella pneumoniae</i>	NT	R	S	S	S	S	S	NT	R
<i>Bordetella parapertussis</i>	S	R	R	S	S	S	S	R	R
<i>Streptococcus pyogenes</i>	S	S	S	S	R	R	S	S	S

Antibiograms for the test pathogens used in this study. Result = Mean±Standard deviation. R = resistant, S = sensitive., NT = not tested. E (Erythromycin, 25µg), AMP (Ampicillin,10µg), CTX (Cefotaxime, 5µg), F (Nitrofurantoin, 300µg), CN (Gentamicin, 10µg), SXT (Sulphamethoxazole/Trimethoprim, 25µg), CIP (Ciprofloxacin, 2.5µg), RD (Rifampicin, 5µg), FOX (Cefoxitin, 10µg).

Table 4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of product E8 and controls against *M. catarrhalis*

EXTRACT	MIC (µg/mL)	MBC (µg/mL)
E8	125	500
Cephalexin	62.5(S)	125
Trimethoprim	15.6(S)	125

E = Extract, S = sensitive

Discussion

Results from this study have shown that product E8 has activity against *Moraxella catarrhalis*. *M. catarrhalis* is known to cause chronic ear infections (otitis media) which is a significant public health issue all over the world particularly because of the associated antibiotic resistance ([22], [23], [24], [25]) mainly due to biofilm formation in the middle ear of infected patients [26]. *M. catarrhalis* may also cause other respiratory infections such as pneumonia, bronchitis, endocarditis, sinusitis and chronic obstructive pulmonary disease (COPD) in adults ([23], [27], [25], [28]. In a recent study at one of the teaching hospitals in Ghana, *M. catarrhalis* was found to be one of the major bacteria isolates in the sputum samples of adult patients hospitalized with cases of community-acquired pneumonia infections. Other pathogens contributing to pneumonia were *Streptococcus species*, *Citrobacter diversus* and *Staphylococcus species* [29].

Extract E8 was prepared by the manufacturer from *Khaya senegalensis*, *Nauclea latifolia*, *Pilostigma thonningii*, *T. monadelph*a and *Clausina anisata*. Ethanol extracts of *K. senegalensis* has been reported to have shown antimicrobial activity against *C. albicans* [30], *E. coli*, *Salmonella typhi* [31], *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Enterobacter species* ([32], [33]). *N. latifolia* alcohol and aqueous extracts exhibited antimicrobial activity against *E. coli*, *Streptococcus pneumoniae*, *Shigella dysenteriae*, *S. aureus* [34], *P. aeruginosa* and *S. typhi* [35]. Methanol and flavonoid extracts of *P. thonningii* was effective against *Strptococcus pyogenes*, *S. dysenteriae* [36] and *S. aureus* [37]. The essential oil and crude extracts of *C. anisata* is reported to have antimicrobial activity against *Neisseria gonorrhoeae* [38], *Cryptococcus neoformans*, *Candida tropicalis*, *Candida krusei* and *Candida parapsilosis* [39]. It is also reported to be active against *Shigella species*, *Proteus species*, *Aspergillus niger* and *Aspergillus parasiticus* [40]. Information in relation to

the antimicrobial activity of *T. monadelph*a has not been reported. None of the plant constituents of E8 has so far been reported to have antimicrobial activity against *M. catarrhalis*. Although individual plant components of the product E8 have been reported to possess antimicrobial activities, the product was inactive against most of the microorganisms tested. This could be due to the different methods used in the preparation of extract (organic vs aqueous) or an antagonistic effect of a plant constituent.

It is now important to determine the medicinal plant constituent(s) of the E8 aqueous extract that are responsible for the observed bactericidal activity against *M. catarrhalis*. After identification of the plant constituents responsible for the antimicrobial activity, it will be highly desirable to compare the performance of the bactericidal function of the aqueous extract by oral versus topical versus intratympanic delivery, initially using a laboratory animal model, best suited for effective treatment of otitis media. This is notwithstanding the opportunity arising from this research for isolation of the relevant bioactive compound(s) permitting standardisation of effective dose of the extract for treatment of infections caused by *M. catarrhalis*.

Conclusion

The role of medicinal plants in the treatment of various disease conditions since time immemorial cannot be overstated. The aim of this study was to screen pre-packaged medicinal plant products commonly sold on the Ghanaian marketplace for their antimicrobial activity. One out of the nine products screened (E8) was significantly active against *M. catarrhalis in vitro*. Generally, most medicinal plant products are produced by combination of two or more plants with the belief that their activity or effect may be enhanced (synergy). This belief cannot be verified until the medicinal plant constituent (s) responsible for the antimicrobial activity is /are identified. Further *in vitro* evaluation of individual plant constituents responsible for antimicrobial activity of extract E8 against *M. catarrhalis* is highly warranted.

Conflict of Interest

The authors declare no conflict of interest

Acknowledgement

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Herbal medicine is widely used, especially in developing countries such as Ghana, where there is a dual system of medical practice which recognises both conventional and herbal medicine. It is estimated that about 951 tons of crude herbal mixture (excluding pre-packed herbal medicine) are sold annually on the Ghanaian domestic market, amounting to a total of US\$7.8 million market value and an export value of US\$15 million (van Andel, Myren and Onselen, 2012). Though herbal medicine has been in use over a long period of time, sufficient scientific data supporting its effect on claimed human ailments is limited. While current research efforts on the therapeutic potential of Ghanaian medicinal plants focus mainly on the screening for phytochemical constituents, antimicrobial efficacy, and toxicity, there is the need to investigate both potential benefits as well as deleterious effect of these herbal medicinal products as these can be inherently dangerous if they contain therapeutic levels of toxic constituents which are not screened for in the manufacturing process.

6.1 Mutagenic Potential of the Medicinal Plant Products

All the Ghanaian medicinal plant product formulations, representing a mixture of aqueous extract of different medicinal plants were found to be non-mutagenic using the three strains (TA98, TA100 and TA1537). However, extract E8 which was prepared by the herbal manufacturer from a combination of *C. anisata*, *P. thonningii*, *T. monadelpha*, *N. latifolia* and *K. senegalensis* showed potential antimutagenic effects *in vitro* against the TA98 and TA100.

Extract E8, that was produced by the herbal manufacturer from an aqueous mixture of *C. anisata*, *N. latifolia*, *K. senegalensis*, *P. thonningii* and *T. monadelpha*, produced a 2-fold decrease in revertant colonies compared to the negative controls in both the with and without metabolic activation systems of TA98 and TA100. Clearly, the antimutagenic activity must be confined to one or more medicinal plants used for the preparation of E8. Aqueous and methanol extracts of *K. senegalensis* have been reported to exhibit 2,2, diphenyl-2picryl-hydrazyl (DPPH) free radical scavenging activity at 1.28 to 21µg/mL (Lompo et al, 2007). A similar antioxidant and free-radical scavenging activity of *K. senegalensis* methanol extract at 500mg/mL has

also been reported (Atawodi et al, 2009). This plant had previously been reported to exhibit anti-cancer activity for human colon cancer, human breast cancer and cervical cancer cell lines at 2-200 parts per million (ppm) (Zhang et al, 2007). On the other hand, an alkaloid rich chloroform fraction isolated from *N. latifolia* was reported to have produced some mutagenicity in the Ames spot test with TA 97 and TA 100 at 10µg. The extract also exhibited an anti-proliferative activity on human monocytes (Traore et al, 2000). Hydro-methanolic saponin fractions isolated from the plant have also been associated with DNA-damage and chromosome mutations in Chinese Hamster ovary cells at 2-10µg (Liu et al, 2011). *Clausena anisata* fraction, chalepin was reported to be non-mutagenic in an Ames spot test at 500µg (Uwaifo, 1984). However, the ethanol extract of *P. thonningii* has been reported to exhibit antioxidant and free radical scavenging activity *in vivo* against carbon tetrachloride-induced hepatic and oxidative damage in Albino rats at 10, 100 and 200mg/kg body weight (Taofeek, 2011). Methanol extract of the plant was also non-toxic at 10-5000µg/mL in a brine shrimp lethality assay (Ajaiyeoba et al, 2006). Extracts of *T. monadelphae*, ethyl acetate, petroleum ether and ethanol also exhibited free radical scavenging activity at 0.1-3mg/mL in a DPPH and reducing power assay (Ben et al, 2013). Although *N. latifolia* is reported to have some mutagenic activity, its toxic effect in E8 appears to have been suppressed potentially by unknown interactions with bioactives produced by the other four plants in the extract. The antimutagenic results obtained from the test could be as a result of the reported antioxidant activities of the four plant components in the mixture. Other studies have shown a correlation between antioxidant and free radical scavenging activity of plants and their antimutagenicity. (Gow-Chin and Hui-Yin, 1995; González-Avila et al, 2003).

Extract E1 was prepared by the herbal manufacturer from an aqueous mixture of *N. latifolia*, *P. fraternus* and *C. sanguinolenta*. Ethanol extract of *C. sanguinolenta* compound, cryptolepine has been reported to be cytotoxic on P388 murine human leukemia cell lines (Dassonnevillu et al, 2000). Cryptolepine also caused DNA damage in human lymphocytes, whilst inducing mutations in chinese hamster lung fibroblast cell lines (Ansah, et al., 2002; Gopalan et al, 2011). *Phyllanthus fraternus* hydro-alcoholic extract is reported to have antioxidant activity on superoxide radicals and alcohol-induced oxidative stress in liver mitochondria of rats (Santos et al,

2000). Aqueous extract of the plant also showed a similar antioxidant activity by altering lipid peroxidation and glutathione levels in oxidative stress rat brain (Naga et al, 2008). On the other hand, *N. latifolia* had earlier chloroform fraction was reported to have produced some mutagenicity in the Ames spot test with TA 97 and TA 100 at 10µg. Furthermore, the fraction exhibited an anti-proliferative activity on human monocytes at the same concentration (Traore et al, 2000). The negative mutagenic results from extract E1 could be attributed to the potential antioxidant activity of a component of the mixture, which suppressed the toxic effect of other plants present in the mixture.

Extract E2 is prepared by the herbal manufacturer from aqueous mixture of *O. gratissimum*, *N. latifolia*, and *C. sanguinolenta*. Methanol leaf extract of *O. gratissimum* showed antimutagenic activity, inhibiting His⁻ to His⁺ reverse-mutation induced by ethyl methanesulfonate against *Salmonella typhimurium* TA100 (Obaseiki-Ebor et al., 1993). Both methanol hexane extracts also showed antioxidant activity by scavenging DPPH free radical (Akinmoladun et al, 2007; Oboh et al, 2008). *Ocimum gratissimum* aqueous extract had inhibitory effect on human breast tumour growth (Hangia-Makker et al, 2007). DNA damaging and cytotoxicity effect of *C. sanguinolenta* has been reported (Ansah et al, 2002; Wright et al, 2001; Miert et al, 2005). *N. latifolia* also reportedly produced mutagenic effect with strains TA 97 and TA 100 (Traore et al, 2000) and DNA damaging with chromosomal mutations in mammalian cells (Liu et al, 2011). The mutagenicity test of extract E2 in this study was negative for all strains of bacterial used. This could be due to the antagonistic effect resulting from the combination of the three plants making them less toxic to the bacterial cells.

Extract E4 was prepared by the herbal manufacturer from a mixture of *C. ferruginea* and *P. pinnata*. The negative mutagenicity results of extract E4 obtained in the Ames test is consistent with reported literature, as none of the plant components had been reported for mutagenicity. *Cnetis ferruginea* methanol extract has been reported to produce negative results in a bacterial reverse mutation assay (Hong and Lyu, 2011) whilst its aqueous leaf extract was also reported by Garon et al (2007) to be

cytotoxic. Methanol, aqueous and ethanol extracts of this plant has also been reported by Akharaiyi et al, (2012) and Oke and Hamburger, (2001) to have antioxidant activity against DPPH free radical. *Paullinia pinnita* methanol extract exhibited DPPH free radical scavenging activity (Annan et al, 2009; Jimoh et al, 2007).

Extract E5 is prepared from an aqueous combination of *M. charantia*, *V. amagdalina*, *S. hispidus* and *L. multiflora*. *Momordica charantia* hexane and aqueous extracts exhibited antioxidant activity whilst reducing carcinogen-induced lipid peroxidation in the liver and DNA damage in lymphocyte (Semiz and Sen, 2007; Sharma et al, 2011). *Vernonia amagdalina* ethyl acetate and petroleum ether extract is reported to have exhibited antimutagenic activity against *S. typhimurium* strains TA100 at 20µg/plate (Obaseiki-Ebor et al, 1993). It also has anticancer activity human adenocarcinoma cells (Yedjou et al, 2008). Furthermore, *Lippia multiflora* methanol exhibited moderate cytotoxic effects on human breast adenocarcinoma MCF-7 and human lung carcinoma COR L23 cell lines (Ajaiyeoba et al, 2006). The combined effect of the four extracts produced a negative results for mutagenicity test. This result is therefore consistent with previous studies (Obaseiki-Ebor et al, 1993). which have not shown any mutagenic effect of any of the plant components of the extract.

Extract E6 was prepared by the herbal manufacturer from combination of aqueous extract of *K. senegalensis*, *K. africana* and *M. stipulosa*. *Khaya senegalensis* methanol has been reported to have anti-cancer activity against human breast cancer, human colon cancer and cervical cancer cell lines (Zhang et al, 2007), whilst the hexane extract of the root and stem-bark had free radical scavenging activity (Atawodi et al, 2009). *Kigelia africana* ethanol extract gave negative results for *S. typhimurium* strains TA98 and TA100 without metabolic activation at 500µg (Kolodziej, 1997). A similar negative results of methanol extract of the plant against TA98 and TA100 at 5, 0.5 and 0.05mg/mL was reported by Elgorashi et al, (2003). Also, triterpene derivative compounds isolated from ethanol extract of *M. stipulosa* had been reported to be cytotoxic (Tapondjou, 2002). The combined effect of plants

in product E6 was negative with or without metabolic activation at all concentrations. This is consistent with previous studies (Elgorashi et al, 2003; Kolodziej, 1997) with strains TA98 and TA100 without metabolic activation.

Extract E7 has *M. myristica*, *C. excelsa*, *A. cordifolia* and *K. senegalensis* as its main component as prepared by the herbal manufacturer. The combined effect of the plants in this mixture gave negative results for mutagenicity for all strains used, which is consistent with results of other aqueous extracts (E1-E9) yielding the same outcome. Methanol extract of *Mitragyna myristica* is reported to have exhibited low cytotoxic activity on MRC-5 human diploid embryonic lung cell (Kekana-Douki et al, 2011). However, the ethanol and methanol extract of this plant has also been reported to have DPPH and hydroxyl free radical scavenging activity (Ogunmoyole et al, 2012; Uyoh et al, 2013). Methanol extract of *A. cordifolia* gave a negative mutagenicity results with strains TA 98, TA100, TA1535, TA1537 and *E.coli* WP2uvrA at 625, 2,500 and 5000µg/plate respectively (Hong and Lyu, 2011). *Chlorophora excelsa* dimethyl formamide extract also gave a negative results in the Ames test for mutagenic or antimutagenic potential for TA97 at 205 and 75mg/ml (Padayachee and Odhav, 2001). *Khaya senegalensis* has earlier been reported to have anti-cancer and antioxidant activity (Atawodi et al, 2009; Kayser and Abreu: Zhang et al, 2007). It appears, therefore, that aqueous extracts of the plant constituents of this herbal medicine formulation do not possess mutagenic or antimutagenic potential.

Extract E9 is composed of three medicinal plants, *V. amygdalina*, *M. erythrophylla* and *A. nobilis* prepared in aqueous form by the herbal manufacturer did not yield any mutagenic or antimutagenic potential. *Vernonia amagdalina* ethyl acetate, methanol and petroleum ether extracts has earlier been reported to have exhibited antimutagenic activity with *S. typhimurium* strains TA98 and TA100 at 200µg/plate (Obaseiki-Ebor et al, 1993). *Anthscleista nobilis* methanol extract is reported to have DPPH free radical scavenging activity (Annan and Dickson, 2008). The negative mutagenicity results observed for E9 may have been due to difference in method of

extraction viz., water versus organic solvent or due to an antagonistic effect of a plant constituent in the cocktail formulation.

Extract E10 was prepared by the herbal manufacturer from prepared *D. adscendens*. This plant is reported to have DPPH free radical scavenging activity well and weakly cytotoxic on A375 melanoma cells (Konan et al, 2012; Mènan et al, 2006; Muanda et al, 2011). *Desmodium adscendens* has not yet been reported to be mutagenic, which is consistent with the results obtained from this study.

In summary, there was no evidence for mutagenic potential for any of the nine aqueous products tested this investigation. However, antimutagenic potential of E8 was evident regardless of the use of native or activated microsomal enzymes prepared from adult pig liver. The specific plant constituent (s) responsible for this activity need to be identified as a potential aid in the treatment of cancer.

6.2 Antimicrobial Activity of the Medicinal Plant Products

Evaluation of the antimicrobial activity test of the Ghanaian medicinal plant product formulations showed that extract E8 possessed antimicrobial activity against *Moraxella catarrhalis*. Due to the significance of *M. catarrhalis* in the aetiology of chronic ear infections (otitis media) and respiratory infections (pneumonia, bronchitis, endocarditis, sinusitis and chronic obstructive pulmonary disease) in adults, further studies aimed at identifying the plant constituent(s) responsible for the observed antimicrobial activity are required (Murphy and Parameswaran, 2009; Cappelletty, 1998, Mikucha, et al., 2000; Ruff, 1998). Studies on the ethanol, methanol or flavonoid extracts of *K. senegalensis*, *N. latifolia*, *P. thonningii*, *T. monadelph*a and *C. anisata*, plant constituents of E8 are also warranted because of their reported broad-spectrum antimicrobial activities against *Streptococcus pyogenes*, *S. dysenteriae* (Akinpelu and Obuotor, 2000), *S. aureus* (Ibewuiké et al., 1997), *C. albicans*, *E. coli*, *Salmonella. typhi*, *Bacillus. subtilis*, *Pseudomonas aeruginosa* and *Enterobacter* species (Abalaka et al. 201; Idu and Igeleke, 2012; Kudi et al. 1999; Oseni and Osman, 2012).

Whilst the essential oil and crude extracts of *C. anisata* are reported to have antimicrobial activity against *Neisseria gonorrhoeae* (Geyid et al. 2005), *Cryptococcus neoformans*, *Candida tropicalis*, *Candida krusei* and *Candida parapsilosis* (Hamza et al. 2006), aqueous extract formulation showed antimicrobial activity against *Aspergillus niger* and *Aspergillus parasiticus* (Osei-safo, 2010).

It is thus clear from the present investigation that different methods for extraction of antimicrobial bioactives will need to be evaluated for antimicrobial activity against pathogens of interest and significance to the community.

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Conclusion and Future Direction

7.1 Conclusions

As stated in the introduction, the aims embodied in this Mphil project were:

- ❖ To screen commonly marketed herbal medicinal products on the Ghanaian market for their mutagenic potential using the bacterial reverse mutation assay (Ames test)
- ❖ To determine the antimicrobial properties of commonly marketed Ghanaian medicinal plant formulations

A. Investigation of the mutagenic potential of the aqueous extracts of the commonly marketed Ghanaian medicinal plant product formulation carried out using Ames test for mutagenicity revealed all tested extracts to be non-mutagenic.

B. Extract E8 which had been produced by the herbal manufacturer from an aqueous mixture of *C. anisata*, *N. latifolia*, *K. senegalensis*, *P. thonningii* and *T. monadelpha*, produced a 2-fold decrease in revertant colonies compared to the negative controls in both the metabolic activation systems of TA98 and TA100, a clear indication of a potential antimutagenic activity, which must be confined to one or more medicinal plants used for the preparation of E8. No investigation was carried out to determine the possible mechanism of antimutagenicity of extract E8. However, it may be attributable to possible antioxidant and/or free radical scavenging activity of one or more of the plant constituents in the herbal formulation

C. The antimicrobial activity of the Ghanaian medicinal plant product formulations, extract E8 was demonstrated to possess significant activity against *Moraxella catarrhalis*, a causative agent of chronic ear infections (otitis media) in infants and other respiratory infections in adults. None of the other extracts showed activity against any of the microorganisms tested. The

minimum inhibitory concentration of E8 was 125µg/well and the minimum bactericidal activity was 500µg/well. It is also important to note that none of the plant constituents of extract E8 has so far been reported to have antimicrobial activity against *M. catarrhalis* (See Chapters 1, section 1.2 and Chapter 5, pages 126-127).

7.2 Future Directions

- ❖ It is recommended that the medicinal plant constituent(s) of E8 aqueous extract responsible for the antimutagenic activity be determined
- ❖ After identification of the plant constituent responsible for mutagenicity, further tests on cytotoxicity should be conducted using a selection of human cancer cell lines to determine the anti-cancer properties of E8.
- ❖ It is important to determine the medicinal plant constituent(s) of the E8 aqueous extract that are responsible for the observed antimicrobial activity against *M. catarrhalis*
- ❖ The route of delivery *in vivo*, of E8 constituent(s) responsible for bactericidal and antimutagenic activities should be determined, initially using appropriate laboratory animal models
- ❖ After validating the antimicrobial and antimutagenic activities of E8 *in vivo*, it will be feasible to identify and isolate the compounds responsible for the antimicrobial and antimutagenic activities for further development as potential antibiotics or drugs for treatment of select carcinomas.

7.3 Study limitations

- ❖ The concentrations of the products used for the mutagenicity test were chosen based on their protein and carbohydrate contents. As such, one low and one high concentration was tested. This was based on the assumption that a product which did not show any activity at a high concentration was unlikely to show any activity at a lower concentration.
- ❖ Only the herbal formulation that showed antimicrobial activity in the agar diffusion test was subjected to further test for MIC/MBC, as is traditionally done.

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APPENDIX A

Reagents used for Preparation of Different types of Growth Media used in this Project.

M9 Salts (5X)

$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$	33.9g/L
KH_2PO_4	15g/L
NH_4Cl	5g/L
NaCl	2.5g/L

56.4g of M9 salts (Sigma-Aldrich, Australia) was dissolved in 1L of Milli Q water, then autoclaved at 121°C and 15psi. Once autoclaved, the M9 salt solution was kept in water bath at $\approx 55^\circ\text{C}$.

1M MgSO_4

12.04g of MgSO_4 was dissolved in 100mL of Milli Q water, then autoclaved at 121°C and 15psi.

1M CaCl_2

11.1g of CaCl_2 was dissolved in 100mL of Milli Q water, then autoclaved at 121°C and 15psi.

20% Glycerol

20mL of glycerol was added to 80mL of Milli Q water, mixed and autoclaved at 121°C and 15psi.

Glucose Minimal Agar (GM)

14g of bacteriological agar No 1 (Oxoid, Australia) was dissolved in 700mL Milli Q water, and then autoclaved 121°C and 15psi. Once the temperature was $\approx 55^\circ\text{C}$, the following sterilised solutions were added:

M9 Salts (5X)	200mL
MgSO_4	2mL
20% Glycerol	20mL
CaCl_2	1 μL

The agar was mixed by swirling upon each addition and contents poured into sterile petri dishes (100X15mm). The plates were allowed to set and sterility was checked by overnight incubation at 37°C.

Top Agar

Bacteriological agar No 1	1.2g
NaCl	1g/L
Milli Q water	200mL

1.2g of bacteriological agar was dissolved in 200mL Milli Q water by heating. 1g of NaCl was then added to the agar mixing to dissolve. The top agar was distributed into 36mL aliquots then autoclaved 121°C and 15psi.

Histidine/Biotin Solution, 0.5mM

D-Biotin	24.8mg
L-Histidine	19.2mg
Milli Q water	200mL

24.8mg of D-Biotin was added to boiling water to dissolve. Then after, 19.2mg of L-Histidine was added to the solution to dissolve. After cooling, the Histidine/Biotin solution was sterilised using 0.22µm filter.

Sodium Phosphate Buffer Solution (0.1mM), pH 7.4

Sodium phosphate monobasic	6.9g
Sodium phosphate dibasic	7.1g

6.9g of sodium phosphate monobasic was dissolved in 500mL of Milli Q water. 7.1g of sodium phosphate dibasic was also dissolved separately in 500mL of Milli Q water. 60mL of the sodium phosphate monobasic solution was added to 440mL solution of sodium phosphate dibasic solution, making a total volume of 500mL and pH adjusted to 7.4 using a pH meter.

Co-factor Solution for S9 Microsome

Co-factor solution for the S9 microsomal fraction was prepared from the following chemicals:

D-Glucose-6-phosphate	0.8g
NADP	1.75g
MgCl	0.9g
KCl	1.35g
Sodium phosphate dibasic	6.4g
Sodium phosphate monobasic	1.4g

These were dissolved in 450mL Milli Q water and sterilised with 0.22µm filter. The solution was stored in 7mL aliquots at -20°C.

Reagents for estimation of carbohydrate content

Phenol Sulphuric acid

5% Phenol	0.5mL
Concentrated H ₂ SO ₄	2.5mL

Reagents for Protein Estimation (Lowry Method)

Biuret reagent

1% CuSO ₄	0.5mL
2% KNaC ₄ H ₄ O ₆	0.5mL
2% Na ₂ CO ₃ in 0.1N NaOH	50mL

Folin-Ciocalteu's Phenol reagent

2N Folin-Ciocalteu's Phenol	1mL
Milli Q water	1mL

Constituents of Nutrient broth

Peptone A	10g/L
Beef extract	10g/L
NaCl	5g/L

Constituents of Mueller-Hinton agar

Beef infusion solid	2g/L
Casein hydrolysate	17.5g/L
Starch	1.5g/L
Agar	17g/L

APPENDIX B

Raw Data of Mutagenicity Experiments

Table B1 - Extract E1

Mutant Strain	Control	Std. Induced	Std. Control	Un-induced		S9 Activated		S9+Rifampin Activated	
		10µg	10µg	1mg	8mg	1mg	8mg	1mg	8mg
TA98	23±0.9	60±0.9	62±1.5	24±0.9	21±1.2	24±1.2	26±0.9	23±0.7	25±1.2
TA100	99±1.2	188±2.1	184±1.8	102±1.5	99±2	99±0.6	104±0.9	108±1.5	115±1.7
TA1537	22±1.3	55±1.5	56±1.2	22±1.2	22±1.5	23±1.5	20±0.6	22±0.6	24±1.5

Results expressed as: Mean ±SEM, Std.: Standard, Standard control: Acridine orange (TA98), Neutral red (TA1537), Sodium azide (TA100), Standard Induced: 2-aminianthracene

Table B2 - Extract E2

Mutant Strain	Control	Std. Induced	Std. Control	Un-induced		S9 Activated		S9+Rifampin Activated	
		10µg	10µg	1mg	8mg	1mg	8mg	1mg	8mg
TA98	23±0.9	60±0.9	62±1.5	27±0.9	25±2.4	26±0.7	24±1.2	26±1.2	31±1.9
TA100	99±1.2	188±2.1	184±1.8	102±1.5	110±0.6	117±2	103±1.5	119±1.9	123±1.2
TA1537	22±1.3	55±1.5	56±1.2	17±1.2	19±1.9	22±0.9	21±1.5	24±1.2	23±1

Results expressed as: Mean ±SEM, Std.: Standard, Standard control: Acridine orange (TA98), Neutral red (TA1537), Sodium azide (TA100), Standard Induced: 2-aminianthracene

Table B3- Extract E4

Mutant Strain	Control	Std. Induced	Std. Control	Un-induced		S9 Activated		S9+Rifampin Activated	
		10µg	10µg	1mg	8mg	1mg	8mg	1mg	8mg
TA98	23±0.9	60±0.9	62±1.5	27±0.9	29±0.9	28±0.3	27±0.6	31±0.9	27±0.6
TA100	99±1.2	188±2.1	184±1.8	119±1.5	125±2	122±1.8	105±1.2	125±2	120±0.9
TA1537	22±1.3	55±1.5	56±1.2	19±1.9	18±1	23±0.6	21±1.5	23±1.2	22±1.3

Results expressed as: Mean ±SEM, Std.: Standard, Standard control: Acridine orange (TA98), Neutral red (TA1537), Sodium azide (TA100), Standard Induced: 2-aminianthracene

Table B4 - Extract E5

Mutant Strain	Control	Std. Induced	Std. Control	Un-induced		S9 Activated		S9+Rifampin Activated	
		10µg	10µg	1mg	8mg	1mg	8mg	1mg	8mg
TA98	23±0.9	60±0.9	62±1.5	25±1	23±0.3	21±1.2	19±0.3	25±1.5	21±1
TA100	99±1.2	188±2.1	184±1.8	120±0.6	124±0.9	121±0.6	101±1.2	123±1.2	121±2.1
TA1537	22±1.3	55±1.5	56±1.2	23±1.2	20±0.9	29±0.9	28±0.6	23±1.2	27±0.6

Results expressed as: Mean ±SEM, Std.: Standard, Standard control: Acridine orange (TA98), Neutral red (TA1537), Sodium azide (TA100), Standard Induced: 2-aminianthracene

Table B5 - Extract E6

Mutant Strain	Control	Std. Induced	Std. Control	Un-induced		S9 Activated		S9+Rifampin Activated	
		10µg	10µg	1mg	8mg	1mg	8mg	1mg	8mg
TA98	23±0.9	60±0.9	62±1.5	20±1.8	22±1.5	29±2.4	23±1.2	32±0.9	29±1.2
TA100	99±1.2	188±2.1	184±1.8	120±0.9	119±0.6	119±1	115±0.6	120±2.5	119±1.9
TA1537	22±1.3	55±1.5	56±1.2	27±1.2	27±1.5	30±0.9	25±0.7	33±0.6	31±0.6

Results expressed as: Mean ±SEM, Std.: Standard, Standard control: Acridine orange (TA98), Neutral red (TA1537), Sodium azide (TA100), Standard Induced: 2-aminianthracene

Table B6 - Extract E7

Mutant Strain	Control	Std. Induced	Std. Control	Un-induced		S9 Activated		S9+Rifampin Activated	
				1mg	8mg	1mg	8mg	1mg	8mg
TA98	43±1.2	86±1.3	97±1.3	27±1.2	27±0.8	30±0.9	25±0.9	33±0.6	30±0.3
TA100	126±1.5	264±0.8	288±1.5	124±1.8	124±1.8	123±1.5	104±2.6	120±0.3	105±2.4
TA1537	21±0.3	51±1.7	56±1.5	24±0.3	31±1.3	24±0.6	29±0.3	26±1	29±1.2

Results expressed as: Mean ±SEM, Std.: Standard, Standard control: Acridine orange (TA98), Neutral red (TA1537), Sodium azide (TA100), Standard Induced: 2-aminianthracene

Table B7 - Extract E8

Mutant Strain	Control	Std. Induced	Std. Control	Un-induced		S9 Activated		S9+Rifampin Activated	
				1mg	8mg	1mg	8mg	1mg	8mg
TA98	43±1.2	86±1.3	97±1.3	34±4.0	30±1.7	35±1.2	19±1.2	36±0.9	12±0.9
TA100	126±1.5	264±0.8	288±1.5	127±4.7	130±1.5	118±1.2	55±2.6	141±0.9	47±1.6
TA1537	21±0.3	51±1.7	56±1.5	25±1.7	17±0.6	28±2.5	23±0.6	17±0.7	13±0.3

Results expressed as: Mean ±SEM, Std.: Standard, Standard control: Acridine orange (TA98), Neutral red (TA1537), Sodium azide (TA100), Standard Induced: 2-aminianthracene

Table B8 - Extract E9

Mutant Strain	Control	Std. Induced	Std. Control	Un-induced		S9 Activated		S9+Rifampin Activated	
				1mg	8mg	1mg	8mg	1mg	8mg
TA98	43±1.2	86±1.3	97±1.3	30±1.5	31±0.8	32±0.8	30±1.7	31±1.7	30±0.9
TA100	126±1.5	264±0.8	288±1.5	122±1.5	96±0.9	102±0.7	98±0.6	107±1.5	103±1.2
TA1537	21±0.3	51±1.7	56±1.5	28±1.5	28±1.2	30±2	21±1.2	24±2	20±0.9

Results expressed as: Mean ±SEM, Std.: Standard, Standard control: Acridine orange (TA98), Neutral (TA1537), Sodium azide (TA100), Standard Induced: 2-aminianthracene

Table B9 - Extract E10

Mutant Strain	Control	Std. Induced	Std. Control	Un-induced		S9 Activated		S9+Rifampin Activated	
				1mg	8mg	1mg	8mg	1mg	8mg
TA98	43±1.2	86±1.3	97±1.3	34±4.0	30±1.7	28±1.2	29±0.9	29±1.2	31±1.2
TA100	126±1.5	264±0.8	288±1.5	120±0	114±0.9	130±0.6	114±3.8	123±1.5	124±1.5
TA1537	21±0.3	51±1.7	56±1.5	29±1.7	30±0	21±2	20±0.6	26±2.7	24±0.7

Results expressed as: Mean ±SEM, Std.: Standard, Standard control: Acridine orange (TA98), Neutral red (TA1537), Sodium azide (TA100), Standard Induced: 2-aminianthracene

Table B1 - Protein Content of Ghanaian Medicinal Plant Extracts

Extract	Dilution	µg/mL	µg/mL x dilution	mg/mL	Total Volume/Bottle (mL)	Total Concentration/ Bottle (mg/mL)
01	1/80	10.8	864	0.864	330	285.12
02	1/80	1.2	96	0.096	350	33.6
04	1/80	21.6	1728	1.728	330	570.24
05	1/80	3.4	272	0.272	500	136
06	1/100	14.2	1420	1.420	330	468.6
07	1/120	22	2640	2.640	500	1320
08	1/100	20.2	2020	2.020	330	666.6
09	1/100	12.2	1220	1.220	500	610
10	1/100	8.0	800	0.8	330	264

Table B2 - Carbohydrate Content of Ghanaian Medicinal Plant Extracts

Extract	Dilution	µg/mL	µg/mL x dilution	mg/mL	Total Volume/Bottle (mL)	Total Concentration/ Bottle (mg/mL)
01	1/80	47	3760	3.76	330	1240.8
02	1/80	21	1680	1.68	350	588
04	1/80	101	8080	8.08	330	2666.4
05	1/80	28	2240	2.24	500	1120
06	1/80	107	8560	8.56	330	2824.8
07	1/80	110	8800	8.80	500	4400
08	1/80	108.5	8680	8.68	330	2864.4
09	1/80	78	6240	6.24	500	3120
10	1/80	36	2880	2.88	330	950.4

Raw data for Carbohydrate and Protein Standard Curves

Table B3 – Carbohydrate standard curve

Glucose concentration	Absorbance at 490nm (\pmSEM)
10	0.0085 \pm 0.00006
20	0.017 \pm 0.0001
30	0.028 \pm 0.0002
40	0.0034 \pm 0.0001
50	0.0446 \pm 0.0002
60	0.0579 \pm 0.0002
70	0.0618 \pm 0.0001
80	0.0724 \pm 0.00005
90	0.0818 \pm 0.0002
100	0.089 \pm 0.0009

Table B4 – Protein standard curve

Protein concentration	Absorbance at 650nm (\pmSEM)
1	0.015 \pm 0.0009
2.5	0.047 \pm 0.001
5	0.089 \pm 0.001
10	0.154 \pm 0.0006
20	0.259 \pm 0.001

Raw data for antimicrobial susceptibility testing of products and antibiogram

Table 2 Sensitivity test results of extracts zones of inhibition (mm)

ORGANISM	E1	E2	E4	E5	E6	E7	E8	E9	E10
<i>Escherichia coli</i>	0	0	0	0	0	0	0	0	0
<i>Candida albicans</i>	0	0	0	0	0	0	0	0	0
<i>Moraxella catarrhalis</i>	0	0	0	0	0	0	20±1.2	0	0
<i>Pseudomonas aeruginosa</i>	0	0	0	0	0	0	0	0	0
<i>Acinetobacter baumannii</i>	0	0	0	0	0	0	0	0	0
<i>Streptococcus agalactiae</i>	0	0	0	0	0	0	0	0	0
<i>Salmonella typhimurium</i>	0	0	0	0	0	0	0	0	0
<i>Klebsiella pneumonia</i>	0	0	0	0	0	0	0	0	0
<i>Bordetella parapertusis</i>	0	0	0	0	0	0	0	0	0
<i>Streptococcus pyogenes</i>	0	0	0	0	0	0	0	0	0

Result = mean±standard deviation. 0 = no inhibition

Table 3 Antibigrams for the test pathogens used in this study

ORGANISM	E	AMP	CTX	F	CN	SXT	CIP	RD	FOX
<i>Escherichia coli</i>	NT	0	25±1	25±1	20±0	30±2	40±1	NT	20±2
<i>Candida albicans</i>	NT	NT	0	0	0	0	0	0	0
<i>Moraxella catarrhalis</i>	27±2	0	10±1.5	36±2	31±1	30±1.5	32±1	23±1.2	25±0
<i>Pseudomonas aeruginosa</i>	NT	0	0	0	16±0	0	28±2	NT	0
<i>Acinetobacter baumannii</i>	16±0	0	0	10±1	19±0.5	0	22±0	0	0
<i>Streptococcus agalactiae</i>	24±0.5	24±1	22±2	24±0	14±1	22±0	16±2	29±0	20±1
<i>Salmonella typhimurium</i>	NT	20±1.5	28±3	25±1	20±0	29±1	36±1	NT	22±0
<i>Klebsiella pneumoniae</i>	NT	0	23±0	20±1	18±0.5	25±3	30±0	NT	10±0
<i>Bordetella parapertussis</i>	25±2	0	0	15±0	30±1	35±0	35±0	0	0
<i>Streptococcus pyogenes</i>	14±0	31±0	31±0.5	28±2	9±0	0	15±0	18±1	25±1

Result = Mean±Standard deviation. Zone of inhibition = mm. E (Erythromycin, 25µg), AMP (Ampicillin, 10µg), CTX (Cefotaxime, 5µg), F (Nitrofurantoin, 300µg), CN (Gentamicin, 10µg), SXT (Sulphamethoxazole/Trimethoprim, 25µg), CIP (Ciprofloxacin, 2.5µg), RD (Rifampicin, 5µg), FOX (Cefoxitin, 10µg).

APPENDIX C

Calculations for Estimation of Cytochrome P450 using the Spectral Assay

Cytochrome P450 concentration is calculated by the following formula (Guengerich et al, 2009).

$(\Delta A_{450} - \Delta A_{490}) / 0.091 = \text{nmol of P450 per ml}$, where 0.091 is the Extinction Coefficient ($\Delta \epsilon_{450-490}$). The final concentration is expressed as P450 per mg protein.

Estimation of P450 in the S9 activated fraction

For S9 activated fraction only, 1/2.5 dilution factor was used in the spectral measurement at the protein concentration of 0.4mg/mL.

$$(\Delta A_{450} - \Delta A_{490}) / 0.091$$

$$(0.01459 - (-0.00078)) = 0.01537$$

$$0.01537 / 0.091 = 0.169 \mu\text{M P450}, \times 2.5 = 0.425 \mu\text{M P450} = 0.425 \text{ nmol/mL}$$

$$1\text{mL} = 0.425 \text{ nmol} / 0.4 \text{ mg protein} = 1.063 \text{ nmol/mg protein}$$

Therefore the P450 concentration of Sample A = 1.063 nmol/mg protein

Estimation of P450 in the S9 fraction activated by Rifampin

For S9+Rifampin activated fraction, 1/2.5 dilution factor was used in the spectral measurement and the protein concentration was 0.3mg/mL.

$$(\Delta A_{450} - \Delta A_{490}) / 0.091$$

$$(0.02040 - 0.00102) = 0.01938$$

$$0.01938 / 0.091 = 0.213 \mu\text{M P450}, \times 2.5 = 0.533 \mu\text{M P450} = 0.533 \text{ nmol/mL}$$

$$1\text{mL} = 0.533 \text{ nmol} / 0.3 \text{ mg protein} = 1.777 \text{ nmol/mg protein}$$

Therefore the P450 concentration of Sample A = 1.777 nmol/mg protein

To Whom It May Concern,

I, **Fidelia Senayah**, as a first author declare myself as the primary contributor to the experimental design, interpretation and writing to the paper/publication entitled "Mutagenic Potential of Commonly Marketed Ghanaian Medicinal Plant Formulations using Bacterial Reverse Mutation assay"



First author signature

I, as a Co-Author, endorse that this level of contribution by the candidate indicated above is appropriate.

Heather Fairhurst

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Co-Author 1 signature

Alain Delhaize

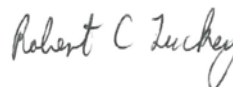
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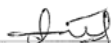
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To Whom It May Concern,

I, **Fidelia Senayah**, as a first author declare myself as the primary contributor to the experimental design, interpretation and writing to the paper/publication entitled "A review of the biological activities and traditional uses of plant constituents in some Ghanaian medicinal products".



First author signature

I, as a Co-Author, endorse that this level of contribution by the candidate indicated above is appropriate.

Trilochan Mukkur
Co-Author



Co-Author signature

To Whom It May Concern,

I, **Fidelia Senayah**, as a first author declare myself as the primary contributor to the experimental design, interpretation and writing to the paper/publication entitled "Antimicrobial Activity of Ghanaian Medicinal Plant Products".


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